PRESCRIBING INFORMATION – Iclusig® (ponatinib) film coated tablets 15 mg, 30 mg or 45 mg ponatinib (as hydrochloride) to be used in the following indications:

**Indications:**

Chronic phase (CP), accelerated phase (AP), or blast phase (BP) chronic myeloid leukemia (CML) in adults who have been intolerant or have failed to respond to prior TKI therapy and for whom subsequent treatment with imatinib, nilotinib, or dasatinib is not clinically appropriate; or who have the T315I mutation.

**Contraindications:**

- Philadelphia chromosome positive acute lymphoblastic leukaemia (Ph+ ALL) who are resistant/intolerant to dasatinib and for whom subsequent treatment with imatinib is not clinically appropriate; or who have the T315I mutation.

**Warnings and precautions:**

- Hypertension: monitor and manage throughout treatment; may increase risk of arterial thrombotic events including renal artery stenosis.
- Severe liver function impairment may result in fatal outcomes.
- Drug-induced liver injury. Hepatic failure (including fatal outcome) has been observed, mostly in first year of treatment.
- Pancreatitis: increased frequency with Iclusig® compared to prior TKI treatments.
- Lipase: check serum lipase fortnightly for 2 months and then periodically.
- Severe renal impairment: may increase the risk of renal-related adverse events including renal artery stenosis.
- Cardiac arrhythmia: QT prolongation: monitor for ECG changes before and during treatment.
- Retinal vein occlusion (RVO) can occur with all TKIs.
- Skin reactions: Stevens-Johnson syndrome and toxic epidermal necrolysis (TEN) have been reported with all TKIs: continue close monitoring with TEN.
- Hypersensitivity reactions: include anaphylaxis and angioedema.
- Pregnancy and breastfeeding: advise patients not to become pregnant or father a child during treatment.
- Drug interactions: see SmPC for details.

**Important ADRs:**

- Most severe events occurred in first 3 months; overall, events occurred more frequently in AP-CML, BP-CML or Ph+ ALL than CP-CML. Caution with use of anti-clotting agents.
- Arterial occlusive events including retinal vein occlusion, peripheral arterial occlusive disease, pancreatitis, pyrexia, abdominal pain, anaemia, angina, chest pain, MI, cerebrovascular accident (CVA), coronary artery disease, peripheral arterial disease, deep vein thrombosis, and increased platelet count; hard end points — hypertension, MI, death, stent, coronary artery disease, cerebrovascular accident, respiratory tract infection, decreased respiratory rate in children, decreased cough in children, cough, oedema of the heart, atrial fibrillation, CCF, sepsis, increased lipase.
- Hypersensitivity reactions: anaphylaxis, angioedema, urticaria and other skin reactions, eosinophilia, eosinophilic pneumonitis, chest pain, pleural effusion and pericardial effusion.
- Pancreatitis and serum lipase: check serum lipase fortnightly for 2 months and then periodically.
- Increased lipase: check serum lipase fortnightly for 2 months and then periodically.
- Diabetes:
- Confirmed in patients treated with Iclusig®: increase in glycosylated haemoglobin (HbA1c).
- Risk factors for diabetes: age, obesity, and family history of diabetes.
- Monitoring is recommended in patients who develop an increased body mass index (BMI) or diabetes.
- Increased frequency of events when used alone or in combination with chloroquine hydroxychloroquine.
- Chloroquine hydroxychloroquine is contraindicated in patients with a history of pancreatitis or alcohol abuse.
- QT prolongation: monitor for ECG changes before and during treatment.
- Retinal vein occlusion (RVO) can occur with all TKIs.
- Hypersensitivity reactions: include anaphylaxis and angioedema.
- Pregnancy and breastfeeding: advise patients not to become pregnant or father a child during treatment.
- Drug interactions: see SmPC for details.

**Dosage and administration:**

- Recommended starting dose 45 mg once daily. Dose reductions to 15 mg for CP-CML and 30 mg for AP-CML, BP-CML or Ph+ ALL are also recommended. Dose modifications, or interruptions, should be considered for patients who achieve a Major Cytogenetic Response; consult the SmPC.
- Discontinue in case of disease progression or severe adverse reactions.
- Monitor for ADRs and hepatic, renal and vascular adverse events and laboratory abnormalities before and during treatment.
- Avoid treatment with Iclusig and strong CYP3A4 inhibitors in combination.

**PREGNANCY AND BREASTFEEDING:**

- Advise female patients to use effective contraception during and for 12 months after treatment.

**REFERENCES:**


**DATE OF PREPARATION:**

April 2017

**LEGAL CATEGORY:**

POM

**SUMMARY OF PRODUCT CHARACTERISTICS (SmPC) see Summary of Product Characteristics (SmPC) before prescribing.**

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Johnson P, United Kingdom
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Word of Welcome

On behalf of the EHA Board and the Scientific Program Committee we are pleased to introduce to you this year’s Abstract Program. The richness of the program is a testament to EHA’s spirit: unity through diversity.

The Scientific Program Committee has compiled an exciting program of Simultaneous Oral and Poster Sessions from close to 2500 submitted abstracts representing all fields of hematology. For the second year, a number of presenters will have the opportunity to pitch their abstract. These Poster pitches are an exciting opportunity to promote basic science and research, and to invite delegates to the poster walks.

The six Best Abstracts will be presented during the Presidential Symposium on Friday afternoon. This will be a session not to miss. During this plenary session EHA is also awarding, for the first time, the best abstracts by trainees in four categories in basic and clinical hematology research. These awardees and the travel grant winners can be found on the next page. YoungEHA are the future of hematology!

The late breaking abstract submission is an integral part of the scientific program. The late breaking submission is intended for abstracts with “hot” data that were not available by the time of the regular submission deadline. Only few abstracts, with the most exciting results are selected for a presentation in the Late Breaking Oral Session on Sunday morning.

A selection of abstracts will be presented during the regular Poster Walks. The Poster Session consists of two parts: the Poster Walk and dedicated Poster Browsing Time. This setup guarantees sufficient time for discussion of the important research presented, so look out for the Poster Walk Moderators in their red baseball caps! There will also be E-posters available on the E-poster screens, for which a specific time is allocated during the Poster Browsing Time at the end of each Walk. The Simultaneous Oral Sessions are spread over three days (Friday to Sunday) providing you with ample opportunity to attend a number of these important sessions.

All posters can be viewed on the E-poster screens from Friday morning to Saturday evening. All the abstracts are also available on the EHA Learning Center, for which you have complimentary access after the congress: learningcenter.ehaweb.org.

On behalf of the EHA Board, the committees and all the people involved in this year’s EHA Congress, we thank you for coming to Madrid and wish you a great meeting.

Shai Izraeli
Chair Scientific Program Committee 22nd Congress
Travel Grant Winners

For this Congress 140 travel grants have been awarded to junior members of EHA, based on the mean score of their abstracts. EHA congratulates the following persons with their travel grants:

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YoungEHA Best Abstract Awards

One of the primary missions of the European Hematology Association is to support young hematology clinicians and researchers. This year we are proud to announce the launching of the YoungEHA Best Abstract Awards. These will be awarded to the highest ranking abstracts in the following four categories: Clinicians or medical students training for a PhD degree, PhD research students, postdoctoral fellows and clinical hematology trainees. We are honored that these outstanding YoungEHA trainees will be presenting during the EHA congress – they are the future of Hematology!

**CLINICAL TRAINEE AWARD**
K C Pawlyn, United Kingdom

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K O Schwartzman, Israel

**PHD RESEARCH STUDENT AWARD**
K JG Barcia Duran, USA

**POSTDOCTORAL RESEARCH TRAINEE AWARD**
K F Vinchi, Italy
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<table>
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</tr>
</thead>
<tbody>
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**Simultaneous sessions I**

- New advances in plasma cell disorders and implications for therapy
  - S100 - S104
  - p. 1
- Aggressive Non-Hodgkin lymphoma - 1st line
  - S105 - S109
  - p. 4
- MRD directed treatment in AML
  - S110 - S114
  - p. 6
- New insights into chronic lymphocytic leukemia biology
  - S115 - S118
  - p. 9
- Pathogenesis of MDS
  - S119 - S123
  - p. 11
- Lymphoma biology
  - S124 - S127
  - p. 13
- Thalassemia
  - S128 - S132
  - p. 15
- AML Biology I: Towards molecular therapies
  - S133 - S136
  - p. 17
- Hematopoiesis, stem cells and microenvironment
  - S137 - S140
  - p. 19
- Gene therapy, cellular immunotherapy and vaccination 1
  - S141 - S145
  - p. 21

**Presidential Symposium**

- Best abstracts
  - S146 - S150
  - p. 23

**Poster sessions I**

- Acute lymphoblastic leukemia - Biology 1
  - P151 - P159
  - p. 26
- Acute lymphoblastic leukemia - Clinical 1
  - P160 - P170
  - p. 30
- Acute myeloid leukemia - Biology 1
  - P171 - P180
  - p. 35
- Acute myeloid leukemia - Biology 2
  - P181 - P190
  - p. 39
- Acute myeloid leukemia - Clinical 1
  - P191 - P199
  - p. 43
- Acute myeloid leukemia - Clinical 2
  - P200 - P207
  - p. 48
- Acute myeloid leukemia - Clinical 3
  - P208 - P215
  - p. 52
- Aggressive Non-Hodgkin lymphoma - 1st line
  - P216 - P225
  - p. 56
- Bone marrow failure syndromes incl. PNH - Biology
  - P226 - P235
  - p. 60
- Chronic lymphocytic leukemia and related disorders - Biology 1
  - P236 - P244
  - p. 65
- Chronic lymphocytic leukemia and related disorders - Clinical
  - P245 - P254
  - p. 69
- Chronic myeloid leukemia - Clinical 1
  - P255 - P263
  - p. 74
- Hematopoiesis, stem cells and microenvironment
  - P264 - P274
  - p. 78
- Hodgkin lymphoma
  - P275 - P283
  - p. 82
- Iron metabolism, deficiency and overload
  - P284 - P294
  - p. 86
- Lymphoma biology
  - P295 - P304
  - p. 91
- Multifaced aspects of bleeding disorders
  - P305 - P312
  - p. 96
- Myelodysplastic syndromes – Clinical 1
  - P313 - P319
  - p. 98
- Myeloma and other monoclonal gammopathies - Biology
  - P320 - P329
  - p. 102
- Myeloma and other monoclonal gammopathies - Clinical 1
  - P330 - P339
  - p. 107
- Myeloma and other monoclonal gammopathies - Clinical 2
  - P340 - P349
  - p. 112
- Myeloproliferative neoplasms - Clinical 1
  - P350 - P359
  - p. 117
- Platelet disorders: Basic
  - P360 - P368
  - p. 122
- Quality of life, palliative care, ethics and health economics 1
  - P369 - P378
  - p. 125
- Stem cell transplantation - Clinical 1
  - P379 - P380
  - p. 130
- Thalassemia
  - P381 - P400
  - p. 135
- Transfusion medicine
  - P401 - P406
  - p. 140
**Simultaneous sessions II**

<table>
<thead>
<tr>
<th>Topic</th>
<th>Pages</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Front-line combinations in multiple myeloma and amyloidosis</td>
<td>S407 - S411</td>
<td>142</td>
</tr>
<tr>
<td>Hodgkin and indolent lymphoma - Clinical</td>
<td>S412 - S416</td>
<td>145</td>
</tr>
<tr>
<td>Biology of MPN, JAK2 and beyond</td>
<td>S417 - S421</td>
<td>148</td>
</tr>
<tr>
<td>Clinical trials including treatment discontinuation in CML</td>
<td>S422 - S426</td>
<td>150</td>
</tr>
<tr>
<td>AML Biology II, Epigenetic targets</td>
<td>S427 - S430</td>
<td>153</td>
</tr>
<tr>
<td>Acquired and inherited platelet disorders</td>
<td>S431 - S435</td>
<td>155</td>
</tr>
<tr>
<td>Acute lymphoblastic leukemia - Biology</td>
<td>S436 - S440</td>
<td>157</td>
</tr>
<tr>
<td>Thrombotic disorders</td>
<td>S441 - S445</td>
<td>160</td>
</tr>
<tr>
<td>Stem cell transplantation - Experimental</td>
<td>S446 - S450</td>
<td>162</td>
</tr>
<tr>
<td>Sickle cell disease, enzymes</td>
<td>S451 - S455</td>
<td>164</td>
</tr>
<tr>
<td>New drugs for rescue in relapsed/refractory multiple myeloma</td>
<td>S456 - S460</td>
<td>167</td>
</tr>
<tr>
<td>Improving prognostication and front-line therapy in chronic lymphocytic leukemia</td>
<td>S461 - S465</td>
<td>170</td>
</tr>
<tr>
<td>Aggressive Non-Hodgkin lymphoma - Relapsed/refractory</td>
<td>S466 - S470</td>
<td>172</td>
</tr>
<tr>
<td>Targeted treatment of AML</td>
<td>S471 - S475</td>
<td>175</td>
</tr>
<tr>
<td>Immunotherapy in ALL</td>
<td>S476 - S480</td>
<td>178</td>
</tr>
<tr>
<td>Biology and disease monitoring in CML</td>
<td>S481 - S485</td>
<td>180</td>
</tr>
<tr>
<td>Prognostic markers and new treatment in MDS</td>
<td>S486 - S490</td>
<td>183</td>
</tr>
<tr>
<td>Stem cell transplantation - Clinical 1</td>
<td>S491 - S495</td>
<td>185</td>
</tr>
<tr>
<td>Bone marrow failure and PNH</td>
<td>S496 - S500</td>
<td>188</td>
</tr>
<tr>
<td>Quality of life, palliative care, ethics and health economics</td>
<td>S501 - S505</td>
<td>190</td>
</tr>
</tbody>
</table>

**Poster sessions II**

<table>
<thead>
<tr>
<th>Topic</th>
<th>Pages</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute lymphoblastic leukemia - Biology 2</td>
<td>P506 - P514</td>
<td>193</td>
</tr>
<tr>
<td>Acute lymphoblastic leukemia - Clinical 2</td>
<td>P515 - P525</td>
<td>196</td>
</tr>
<tr>
<td>Acute myeloid leukemia - Biology 3</td>
<td>P526 - P530</td>
<td>201</td>
</tr>
<tr>
<td>Acute myeloid leukemia - Biology 4</td>
<td>P531 - P535</td>
<td>205</td>
</tr>
<tr>
<td>Acute myeloid leukemia - Clinical 5</td>
<td>P536 - P540</td>
<td>209</td>
</tr>
<tr>
<td>Acute myeloid leukemia - Biology</td>
<td>P541 - P545</td>
<td>213</td>
</tr>
<tr>
<td>Aggressive Non-Hodgkin lymphoma - Relapsed/refractory</td>
<td>P546 - P550</td>
<td>217</td>
</tr>
<tr>
<td>Bone marrow failure syndromes incl. PNH - Clinical</td>
<td>P551 - P555</td>
<td>221</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia and related disorders - Biology 2</td>
<td>P556 - P560</td>
<td>223</td>
</tr>
<tr>
<td>Chronic myeloid leukemia - Biology</td>
<td>P557 - P561</td>
<td>227</td>
</tr>
<tr>
<td>Chronic myeloid leukemia - Clinical 2</td>
<td>P562 - P566</td>
<td>231</td>
</tr>
<tr>
<td>Enzymes and sickle cell disease</td>
<td>P567 - P571</td>
<td>235</td>
</tr>
<tr>
<td>Gene therapy, cellular immunotherapy and vaccination</td>
<td>P572 - P576</td>
<td>241</td>
</tr>
<tr>
<td>Indolent Non-Hodgkin lymphoma - Clinical</td>
<td>P577 - P581</td>
<td>245</td>
</tr>
<tr>
<td>Infectious diseases, supportive care</td>
<td>P582 - P586</td>
<td>249</td>
</tr>
<tr>
<td>Myelodysplastic syndromes - Biology</td>
<td>P587 - P591</td>
<td>253</td>
</tr>
<tr>
<td>Myelodysplastic syndromes - Clinical 2</td>
<td>P592 - P596</td>
<td>258</td>
</tr>
<tr>
<td>Myeloma and other monoclonal gammopathies - Clinical 3</td>
<td>P597 - P601</td>
<td>262</td>
</tr>
<tr>
<td>Myeloma and other monoclonal gammopathies - Clinical 4</td>
<td>P602 - P606</td>
<td>266</td>
</tr>
<tr>
<td>Myeloproliferative neoplasms - Biology</td>
<td>P607 - P611</td>
<td>271</td>
</tr>
<tr>
<td>Other Non-malignant hematopoietic disorders</td>
<td>P612 - P616</td>
<td>275</td>
</tr>
<tr>
<td>Platelet disorders: Clinical</td>
<td>P617 - P621</td>
<td>279</td>
</tr>
<tr>
<td>Quality of life, palliative care, ethics and health economics 2</td>
<td>P622 - P626</td>
<td>285</td>
</tr>
<tr>
<td>Stem cell transplantation - Clinical 2</td>
<td>P627 - P631</td>
<td>290</td>
</tr>
<tr>
<td>Stem cell transplantation - Experimental</td>
<td>P632 - P636</td>
<td>295</td>
</tr>
<tr>
<td>Thrombotic disorders</td>
<td>P637 - P641</td>
<td>299</td>
</tr>
<tr>
<td></td>
<td>P642 - P646</td>
<td>304</td>
</tr>
<tr>
<td></td>
<td>P647 - P651</td>
<td>307</td>
</tr>
</tbody>
</table>
# TABLE OF CONTENTS

## Simultaneous sessions III

- Targeted therapies in relapsed in chronic lymphocytic leukemia
- Follicular lymphoma - Clinical
- Changing the strategy of therapy in multiple myeloma
- Old and new drugs in MPN
- Childhood and more intensive treatment of AML
- Stem cell transplantation - Clinical 2
- Biomarkers in ALL
- Infectious diseases, supportive care
- Iron: Deficiency and overload
- Gene therapy, cellular immunotherapy and vaccination 2

## E-posters

- Acute lymphoblastic leukemia - Biology
- Acute lymphoblastic leukemia - Clinical
- Acute myeloid leukemia - Biology
- Acute myeloid leukemia - Clinical
- Aggressive Non-Hodgkin lymphoma - Clinical
- Bleeding disorders (congenital and acquired)
- Bone marrow failure syndromes incl. PNH - Clinical
- Chronic lymphocytic leukemia and related disorders - Biology
- Chronic lymphocytic leukemia and related disorders - Clinical
- Chronic myeloid leukemia - Biology
- Chronic myeloid leukemia - Clinical
- Enzymopathies, membranopathies and other anemias
- Gene therapy, cellular immunotherapy and vaccination
- Hematopoiesis, stem cells and microenvironment
- Hodgkin lymphoma - Clinical
- Indolent Non-Hodgkin lymphoma - Clinical
- Infectious diseases, supportive care
- Iron metabolism, deficiency and overload
- Myelodysplastic syndromes - Biology
- Myelodysplastic syndromes - Clinical
- Myeloma and other monoclonal gammopathies - Biology
- Myeloma and other monoclonal gammopathies - Clinical
- Myeloproliferative neoplasms - Biology
- Myeloproliferative neoplasms - Clinical
- Non-Hodgkin & Hodgkin lymphoma - Biology
- Other Non-malignant hematopoietic disorders
- Platelets disorders
- Quality of life, palliative care, ethics and health economics
- Sickle cell disease
- Stem cell transplantation - Clinical
- Stem cell transplantation - Experimental
- Thalassemias
- Thrombosis and vascular biology
- Transfusion medicine

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S769 - S773 p. 311
S774 - S778 p. 313
S779 - S783 p. 317
S784 - S788 p. 319
S789 - S793 p. 322
S794 - S798 p. 325
S799 - S803 p. 327
S804 - S808 p. 330
S809 - S813 p. 333
S814 - S818 p. 335
e819 - e834 p. 338
e835 - e863 p. 344
e864 - e905 p. 356
e906 - e950 p. 372
E951 - E973 p. 391
E974 - E979 p. 401
E980 - E988 p. 403
E989 - E1015 p. 406
E1016 - E1040 p. 417
E1041 - E1050 p. 428
E1051 - E1073 p. 432
E1074 - E1082 p. 443
E1083 - E1098 p. 447
E1099 - E1118 p. 452
E1119 - E1127 p. 460
E1128 - E1142 p. 464
E1143 - E1153 p. 470
E1154 - E1165 p. 474
E1166 - E1178 p. 478
E1179 - E1199 p. 484
E1200 - E1238 p. 493
E1239 - E1306 p. 507
E1307 - E1319 p. 536
E1320 - E1352 p. 541
E1353 - E1409 p. 557
E1410 - E1429 p. 579
E1430 - E1456 p. 585
E1457 - E1480 p. 595
E1481 - E1495 p. 604
E1496 - E1565 p. 610
E1566 - E1569 p. 638
E1570 - E1589 p. 640
E1590 - E1604 p. 646
E1605 - E1610 p. 651
The best abstracts selected from the late breaking abstract submission are presented during this oral session.

A complete session overview is available via the mobile app or the online program at ehaweb.org
New advances in plasma cell disorders and implications for therapy

S100
NEXT GENERATION SEQUENCING METHODOLOGY FOR DETERMINING CYTOGENETIC RISK STATUS IN THE DARATUMUMAB PHASE 3 CASTOR AND POLLUX STUDIES IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA
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Background: Cytogenetic risk status in multiple myeloma (MM) studies is traditionally determined by using fluorescence in situ hybridization (FISH) or karyotyping to assess chromosomal abnormalities. However, these technologies have limited resolution and a narrow target range, and reproducible interpretation may be confounded by inter-laboratory variation.

Aims: To describe the NGS methodology used to determine cytogenetic risk status in the daratumumab phase 3 CASTOR and POLLUX studies in RRMM.

Methods: Bone marrow aspirates were collected at screening and assessed centrally via NGS. Whole exome sequencing (exome-seq) and RNA sequencing (RNA-seq) was performed using the Illumina HiSeq platform to identify the presence or absence of defined risk markers: t(4;14), t(14;16), or del17p. The use of RNA-seq allowed for investigation of chromosomal translocations in expressed genomic locations at a higher resolution than FISH, and exome-seq data was used to derive the copy number status in coding regions across the genome. RNA-seq was performed using total RNA and rRNA removal to capture translocations involving coding and intronic regions. Translocation calls were made using two fusion callers, and gene expression was quantified to allow for evaluation of genes associated with translocation events. For t(14;14) translocations, the detected events involved RNA-seq reads fused between IgH and WHSC1 or FGFR3. For t(14;16), the detected translocations involved IgH and WWOX. Manual inspection of patients with t(4;14) showed higher WHSC1 or FGFR3 expression, whereas t(14;16) patients showed higher MAF and CCND2 expression. For del17p detection, exome data of each tumor was compared against 100 peripheral blood mononuclear cell (PBMC) control samples from CASTOR and POLLUX studies. Copy number variation data from two callers were combined to utilize information on relative read depth, systematic biases (observed in pooled normal controls), as well as SNP allele frequency (indicative of loss of heterozygosity events). A del17p event was detected when >50% of the 17p region was deleted.

Results: Based on the RNA-Seq and exome results, cytogenetic risk status in the CASTOR and POLLUX studies was defined as high risk with either t(4;14), t(14;16), or del17p, and standard risk with the confirmed absence of these molecular abnormalities. Comparisons of NGS with FISH showed high concordance for t(4;14), t(14;16), and del17p in both studies (Table 1). PFS analyses investigating differences between treatment groups and between risk groups using FISH-derived risk and NGS-derived risk showed consistent results between FISH and NGS, with improvements in PFS being associated with the addition of daratumumab to standard-of-care regimens in both high- and standard-risk subgroups (Figure 1).

Summary/Conclusions: These studies represent the first, comprehensive use of NGS in global phase 3 clinical trials in RRMM. The NGS methodology accurately identified the presence of defined risk populations t(4;14), t(14;16), and del17p and showed good concordance with FISH. As FISH was performed locally with different probes and pathologists, the high degree of concordance between FISH and NGS is notable and supports the use of NGS for determining cytogenetic risk in patients with RRMM. The utility of NGS in these clinical studies extends far beyond the detection of cytogenetic abnormalities and additional analysis are planned to interrogate these datasets in the identification of novel biomarkers.
significantly improved progression-free survival (PFS) and achieved higher overall response rates (ORRs) compared with the respective standard-of-care regimen alone (Dimopoulos MA et al., N Engl J Med 2016;375(14):1319-1331; Palumbo A et al., N Engl J Med 2016;375(8):754-766.). Due to its novel mechanisms of action, addition of D to standard-of-care regimens may benefit RRMM patients who have poor prognoses resulting from high-risk cytogenetic abnormalities.

**Aims:** To examine the efficacy of DRd and Dvd in RRMM patients with standard or high cytogenetic risk status.

**Methods:** Bone marrow aspirates were collected at screening visits from 311/569 patients from POLLUX and from 353/498 patients from CASTOR, and cytogenetic abnormalities were detected via next-generation sequencing (NGS). Patients were considered to be of high cytogenetic risk if they had ≥1 of the following abnormalities: t(4;14), t(14;16), or del17p; patients were considered to be of standard cytogenetic risk if they lacked these abnormalities. Minimal residual disease (MRD) was assessed at suspected complete response (CR) at 3 sensitivity thresholds (10⁻³, 10⁻⁴, and 10⁻⁵) using the ClonoSEQ™ NGS-based assay (Adaptive Biotechnologies, Seattle, WA). Efficacy analyses included PFS, ORR, and MRD-negative rates.

**Results:** For POLLUX, the median follow-up was 17.3 months. Treating high-risk patients with DRd significantly prolonged median PFS vs Rd (top panel Figure 1) and numerically increased ORR (85% vs 67%; P=0.14). Responses to DRd vs Rd included CR or better in 33% vs 6% of these patients, and very good partial responses (VGPR) or better in 63% vs 31%. In standard-risk patients, DRd vs Rd also resulted in significant improvements in median PFS (Figure 1) as well as ORR (95% vs 82%; P=0.0020). Responses to DRd vs Rd included CR or better in 52% vs 24% of these patients, and VGPR or better in 84% vs 51%. At 10⁻⁵ sensitivity threshold, MRD-negative rates for DRd vs Rd were 18% vs 0% (P=0.0027) among high-risk patients and 30% vs 10% (P=0.0001) for standard-risk patients. For CASTOR, the median follow-up was 13.0 months. Treating both high- and standard-risk patients with Dvd vs Vd significantly prolonged median PFS (bottom panel Figure 1) and increased ORR (high risk: 82% vs 62%; P=0.039; standard risk: 85% vs 64%; P=0.0003). Responses to Dvd vs Vd among high-risk patients included CR or better in 30% vs 9% of patients and VGPR or better in 84% vs 34%; among standard-risk patients, responses included CR or better in 25% vs 8% of patients and VGPR or better in 84% vs 27%. At 10⁻⁵ sensitivity threshold, MRD-negative rates for Dvd vs Vd were 14% vs 0% (P=0.0018) among high-risk patients and 12% vs 2% (P=0.0011) for standard-risk patients.

**Summary/Conclusions:** Adding D to Rd or Vd improved treatment outcomes irrespective of cytogenetic risk status in patients with RRMM. Both DRd and Dvd appear to benefit RRMM patients who have poor prognoses due to high-risk cytogenetic abnormalities. Updated data, including analyses based on individual cytogenetic abnormalities, will be presented at the meeting based on longer follow-up.

**S102**

**MINIMAL RESIDUAL DISEASE BY MULTIPARAMETER FLOW CYTOMETRY IN TRANSPLANT ELIGIBLE PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA: RESULTS FROM THE EMM02/HO095 PHASE 3 TRIAL**

**S. Oliva1,2, D. Hofste op Brunnink1,2, L. Rihova3, S. Spada1, B. van der Holt4, R. Troia1, M. Gambella1, L. Pantani4, S. Grammatico5, M. Gilestro1, M. Offidani5, R. Ribolla3, M. Galli5, R. Hajek6, A. Palumbo7, M. Cavo5, P. Omedè1, V. van der Velden8, M. Boccadoro1, P. Sonneveld2

**Background:** Multiple myeloma (MM) is still an incurable disease and patients may relapse despite achievement of complete remission (CR). Available data show that MRD detection is a sensitive strategy to appropriately measure response in MM patients.

**Aims:** We evaluated MRD by MFC in patients with newly diagnosed MM enrolled in the EMM02/HO095 phase 3 trial.

**Methods:** Patients were ≥65 years of age and treatment consisted of Bortezomib-Cyclophosphamide-Dexamethasone (VCD) induction, mobilization and stem cell collection, intensification with Bortezomib-Melphalan-Prednisone (VMP) vs High-Dose-Melphalan (HDM) followed by stem cell transplant, consolidation with Bortezomib-Lenalidomide-Dexamethasone (VRD) vs no consolidation, and Lenalidomide maintenance. MRD was assessed in patients achieving at least a very good partial response (VGPR) before starting maintenance (after HDM, VMP or VRD) and during maintenance every 6-12 months; samples were centralized to 3 European labs. MFC was performed on bone marrow according to Euroflow-based methods (8 colors, 2 tubes) with a sensitivity of 10⁻⁵. Quality checks were done to compare sensitivity and to show correlation between protocols (Hofste op Brunnink D, ASH 2016 abstract 2072).

**Results:** A total of 316 patients could be evaluated before maintenance: median age was 57 years (IQR: 52-62), 18% (57/316) had ISS III and 22% (70/316) had high risk cytogenetic abnormalities defined as presence of either one of the following: t(11;14), t(14;16) or t(14;20); 63% (199/316) had received HDM and 37% (117/316) VMP; thereafter 51% (160/316) had received VRD. After a median follow-up of 30 months from MRD enrolment, 76% (239/316) patients were MRD-negative: 64% (153/239) in the HDM vs 36% (86/239) in the VMP groups. The 3-year PFS was 50% in MRD-positive vs 77% in MRD-negative patients (HR 2.87, 95% CI: 1.75 - 4.72; p<0.001). Subgroup analyses were carried out to assess the risk factors for MRD-positivity according to baseline characteristics and therapies: high risk cytogenetic abnormalities were the most important risk factors (HR 9.87, 95% CI: 4.3 - 22.63; interaction p=0.001). Finally, 48% of MRD positive patients at pre-maintenance who had a second MRD evaluation after at least 1 year of lenalidomide became MRD-negative.

**Summary/Conclusions:** MRD by MFC is a strong prognostic factor in MM patients receiving intensification with novel agents or transplant; lenalidomide maintenance further improved depth of response; high risk cytogenetic abnormalities are the most important prognostic factors in MRD-positive patients.

**S103**

**PHASE I, OPEN-LABEL TRIAL OF ANTI-BCMA CHIMERIC ANTIGEN RECEPTOR T CELLS IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA**

**W. Zhang1,2, W. Zhao1, J. Liu1, A. He1, Y. Chen1, X. Cao1, N. Yang1, B. Wang1, P. Zhang1, Y. Zhang1, F. Wang1, B. Lei1, L. Gu1, Y. Yang1, J. Bai1, R. Zhang1, X. Wang1, X. Ma1, J. Wang1, J. Wang1, L. Wei1, J. Zhang1, X. Zang1, G. Zhuang2, F.X. Fan2

**Background:** Immunotherapy has emerged as a potentially curative treatment in hematological malignancies. Uniformly expressed in plasma cells, B-cell maturation antigen (BCMA) is an appropriate target antigens for CAR T-cell therapies in multiple myeloma.

**Aims:** This phase I, open-label trial was conducted to assess the efficacy and
safety profile of LCAR-B38M anti-BCMA CAR T cells in patients with relapsed/refractory multiple myeloma.

**Methods:** All patients underwent leukapheresis to obtain peripheral blood mononuclear cells and their T cells were engineered to express anti-BCMA CAR. Three doses of 300 mg/m² cyclophosphamide were administered on day -5, -4, and -3 (before recruitment, patients took the same chemotherapy to identify if they were refractory to cyclophosphamide monotherapy) and engineered-T cells were reinfused on day 0, 2, and 6. This trial was divided into the dose escalation stage and expansion cohort. Toxicity and responses were assessed according to the Common Terminology Criteria for Adverse Events (version 4.0) and International Myeloma Working Group (IMWG) Uniform Response Criteria for Multiple Myeloma, respectively.

**Results:** As of the February 20th, 2017 data cut-off, 22 patients had been enrolled, two of whom were diagnosed as plasma cell leukemia. The male:female ratio was 11:11 and median age was 53.5 years. Chromosomal abnormalities were detectable by FISH in eight patients, two of whom involved in the deficiency of p53. Eleven patients were triple refractory (chemotherapy, proteasome inhibitors, and immunomodulatory drugs), 11 resisted to double prior treatments (chemotherapy and proteasome inhibitors/immunomodulatory drugs), and four relapsed after autologous hematopoietic stem cell transplant. The median number of infused CAR T cells was 4.0×10^6 (range, 1.5×10^5-7.0×10^6) per kg. The median follow-up was 131.5 (range, 29-327) days. 100% of patients achieved an objective response. The first six patients achieved complete responses with flow MRD-negative; 14 patients achieved very good partial responses; one patient, with renal failure, achieved partial response; all these 22 patients had kept their best response at the end of follow-up. The pictures we enclosed were the subcutaneous nodules in one patient with extramedullary plasmacytoma. We found that the nodules were obviously decreased after the infusion and disappeared finally. Another one achieved transient partial response, which last for 12 days. He then took the secondary infusion but failed since the post-operation large-dermatitis and the number of previous PCD treatments.

**Summary/Conclusions:** Our findings demonstrated the safety and antmyeloma activity of LCAR-B38M anti-BCMA CAR T cells.

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**S104**

**PATIENTS WITH LIGHT CHAIN AMYLOIDOSIS TREATED WITH NEO001 ACHIEVE RAPID ORGAN RESPONSES THAT ARE INDEPENDENT OF PREVIOUS PLASMA CELL–DIRECTED THERAPIES**


1Mayo Clinic, Rochester, 2Tufts Medical Center, Boston, 3Memorial Sloan Kettering Cancer Center, New York, 4Boston University School of Medicine and Boston Medical Center, Boston, 5University of Pennsylvania, Philadelphia, 6Karmanos Cancer Institute, Detroit, 7JW Consulting, Hillsborough, 8Prothena Biosciences Inc, South San Francisco, 9Stanford University School of Medicine, Stanford, United States

**Background:** Light chain (AL) amyloidosis is a rare and often fatal disease caused by the accumulation of misfolded light chain (LC) aggregates that can lead to progressive failure of critical organs, causing significant morbidity and mortality. Patients' survival depends upon rapid suppression of the misfolded LC and stabilization or recovery of organ function. Current therapies limit LC production; however, ~75% of patients have persistent organ dysfunction. NEO001 is a novel investigational monoclonal antibody that targets misfolded LC and may neutralize circulating LC aggregates and clear insoluble deposits.

**Aims:** To assess the association between responses and time, depth, number or type of previous plasma cell–directed (PCD) treatments and organ response.

**Methods:** Inclusion criteria for this trial were: completed ≥1 PCD treatment before enrollment, attained partial hematologic response (HR) or better to any previous therapy, and have persistent organ dysfunction. NEO001 was administered intravenously every 28 days. During the dose-escalation phase, 27 patients received NEO001 at 0.5, 1, 2, 4, 5, 16, or 24 mg/kg in a 3+3 study design. In the expansion phase, 42 additional patients with renal, cardiac, or nerve involvement were enrolled and treated (24 mg/kg). We assessed cardiac and renal best responses based on consensus criteria. Peripheral nervous system (PN) responses were assessed at month 10 (after 9 infusions) using the Neuropathy Impairment Score—Lower Limbs (NIS-LL). We explored the potential impact on organ response of the number and type of organs affected and the number of, type of, and time since previous therapies at baseline.

**Results:** In the overall population (N=69), the median age was 61 years (61% male). Median (range) time since diagnosis was 2.9 (0.4-16.0) years, and 45% of patients underwent ≥3 previous PCD regimens. Median time to first best response was 1.8 (cardiac), 3.7 (renal), and 1.0 (PN) months. Best response rate indicating organ response was observed in 53% of cardiac-evaluable patients (n=19/36) and 64% of renal-evaluable patients (n=23/36). PN responses were observed in 82% (n=9/11) of PN-evaluable patients. Time from patients' best HR to previous PCD treatment was not related to the attainment of NEO001 organ response (responder/stable: 35.6/36.6 months [cardiac] and 30.6/32.5 months [renal]; P>0.05). Depth of patients' best HR also was not related to the attainment of NEO001 organ response (percentage of patients with organ response in CR/VPR/PR after PCD: 47.1/66.7/42.9% [cardiac] and 68.6/63.6/62.5% [renal]; P>0.05). Similarly, time or depth of patients' last HR did not impact the NEO001 organ response rate (P>0.05). Patients with NEO001 organ responses were no more likely to have had their last PCD therapy <6 than ≥6 months from their first NEO001 dose. Patients' previous PCD treatment type was not related to the corticosteroid for spinal meningioma. He terminally died finally. Another one achieved transient partial response, which last for 12 days. He then took the secondary infusion but failed since the post-operation large-dermatitis and the number of previous PCD treatments.

**Summary/Conclusions:** NEO001 specifically targets disease-causing, misfolded LC aggregates in AL amyloidosis. Organ responses in patients treated with monthly NEO001 infusions were achieved rapidly and independently of time since previous chemotherapy, depth of hematologic response, or predominant type of PCD treatment.
S105
RITUXIMAB MAINTENANCE AFTER AUTOLOGOUS TEM CELL TRANSPLANTATION PROLONGS SURVIVAL IN YOUNGER PATIENTS WITH MALT CELL LYMPHOMA: FINAL RESULTS OF THE LYMA TRIAL OF THE MABEASE STUDY


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Background: Intravenous (IV) rituximab plus chemotherapy is standard treat- ment for diffuse large B-cell lymphoma (DLBCL). A subcutaneous (SC) formu- lation of rituximab may simplify treatment and reduce burden.

Aims: MabEase (NCT01649856) studied efficacy, safety and patient (pt) sat- isfaction with rituximab SC or IV plus CHOP as first-line DLBCL treatment.

Methods: Pts were randomized 2:1 to rituximab SC (IV 375mg/m2 cycle 1; SC 1400mg cycles 2–8) or IV (375mg/m2 cycles 1–8) plus CHOP every 14 or 21 days. Primary endpoint was EFS at 2 years. Secondary endpoints included safety, survival, treatment satisfaction (Cancer Treatment Satisfaction Questionnaire [CTSQ]), Rituximab Administration Satisfaction Questionnaire [RASQ]) and time savings. Follow-up continued until at least 24 months after EOI in the last patient recruited.

Results: Of 576 pts (381 SC; 195 IV), 572 (378 SC; 194 IV) received treatment. EOI CR/Cru rates were 50.6% (95% CI 45.3–55.9) and 42.4 (95% CI 35.1–49.7) in the SC and IV groups, respectively (Table 1). After 35 months’ median follow-up, median progression-free survival (PFS), event-free survival (EFS) and overall survival (OS) were not reached in either arm and no statistically significant differences were observed between treatment arms. PFS, EFS and OS rates were also similar at 24 months’ follow-up (non-significant differences; Table 1). Grade 3 adverse events (58.3% SC; 54.3% IV) and administration-related reactions (21% in both groups) were similar between arms. Of SC recipients, 5.7% had treatment-induced PN grade 2/3, 2.5% in IV; grade 4 PN occurred in 1.5% (IV) and 0.5% (SC) of pts, respectively. PN decreased with SC (43% vs 56% in IV; p=0.020). RASQ scores for ‘impact on activity of daily living’, ‘convenience’ and ‘satisfaction’ were improved with SC vs IV; CTSQ scores were similar between arms (Figure 1).

References
When pts in the SC group were asked, if given the option, which treatment they would prefer, 90.8% stated a preference for SC over IV. Median administration time (6 minutes SC vs 2.6–3.0 hours IV) and chair/bed and overall hospital times were shorter with SC than with IV treatment.

Table 1. Efficacy endpoints in the intent-to-treat population.

<table>
<thead>
<tr>
<th>Efficacy endpoint</th>
<th>Rituximab SC plus CHOP</th>
<th>Rituximab IV plus CHOP</th>
</tr>
</thead>
<tbody>
<tr>
<td>%FV/Relapse-free</td>
<td>N (%[95%CI])</td>
<td>N (%[95%CI])</td>
</tr>
<tr>
<td>CR1</td>
<td>342 (0.60 [0.53–0.67])</td>
<td>342 (0.56 [0.50–0.62])</td>
</tr>
<tr>
<td>PR</td>
<td>342 (0.36 [0.27–0.45])</td>
<td>342 (0.35 [0.27–0.44])</td>
</tr>
<tr>
<td>OR</td>
<td>342 (0.66 [0.58–0.74])</td>
<td>342 (0.61 [0.53–0.69])</td>
</tr>
<tr>
<td>INI</td>
<td>342 (0.70 [0.63–0.77])</td>
<td>342 (0.69 [0.62–0.76])</td>
</tr>
<tr>
<td>Median read depth</td>
<td>743 (576–1027)</td>
<td>743 (595–1175)</td>
</tr>
<tr>
<td>Average transcripts/ million (TPM)</td>
<td>2,256 (with a median TPM of 743)</td>
<td>2,256 (with a median TPM of 743)</td>
</tr>
</tbody>
</table>

Figure 1. Patient satisfaction at cycle 3 and cycle 7 of treatment.

Summary/Conclusions: Rituximab SC had similar efficacy and safety to the IV form, with improvements in patient satisfaction ratings, and administration/hospital time savings. Our findings support the use of rituximab SC in this setting.

S108 ANALYSIS AND CHARACTERIZATION OF HEMATOLOGIC CANCERS USING A COMPREHENSIVE NGS PANEL COMPRISED OF DNA AND RNA BAITS TARGETING 704 GENES

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Background: As next-generation sequencing (NGS) methodologies improve, so does the ability to characterize hematopoietic and lymphoid neoplasms. This promises to revolutionize oncology, allowing more accurate and precise classification of patients and potentially leading to novel targeted and combination therapies with improved outcomes.

Aims: We constructed a custom targeted sequencing panel, MyHEME™, to comprehensively identify and characterize DNA and RNA changes in a broad range of hematologic malignancies, including Non-Hodgkin lymphoma (NHL).

Methods: The MyHEME targeted sequencing panel is comprised of two independent bait sets that target a combined 704 genes known or predicted to contribute to hematologic cancers (DNA baits for 571 genes and RNA baits for 361 genes; 228 genes are found in common between the two bait sets). Libraries were constructed using 1μg of DNA or 0.1μg of RNA and sequenced on an Illumina platform. Sequenced reads are analyzed using proprietary MyInformatics™ software to identify single nucleotide variants (SNVs), indels and structural variants (SVs). Both the MyHEME panel and MyInformatics software were created under ISO13485 design control. To characterize the performance metrics of the MyHEME panel, we used the NIST human reference sample NA12878 along with combinations of hematologic cancer derived cell lines with known pathogenic variants at various allelic frequencies.

Results: Analytical validation of the MyHEME panel established an average read depth of 1,175x (with a median read depth of 1,088x) for the DNA targets and an average transcripts per million (TPM) of 2,256 (with a median TPM of 743) for the RNA targets. For the DNA targets, we established sensitivity >95% (99.8% for SNVs at a 2.5% LOD) and specificity >95% (95.5% for SNVs at a 2.5% LOD; 97.7% for coding indels at a 5.0% LOD). We also show the ability to cross-confirm results between the 228 genes common to both the DNA and RNA targets. Importantly, novel gene fusions, which are generally difficult to detect and validate, were cross-confirmed when observed in both the DNA and RNA targets. For example, we identified a novel t(9:22) translocation causing a NUP214-XXR3 gene fusion using both the DNA and RNA targets. Additionally, while RNA data provides the fused exons of the transcripts, DNA data gives the precise genomic breakpoint coordinate.

Summary/Conclusions: MyHEME is an extensive panel for sensitively and specifically identifying SNV, indel and SV mutations in 704 target genes. This panel can comprehensively characterize mutations in multiple diverse hematologic cancer samples, including Non-Hodgkin lymphoma (NHL), ALL, AML and Multiple Myeloma. By utilizing a high depth of coverage, MyHEME can accurately detect clones present down to 5% of a patient’s sample. In addition, by targeting both DNA and RNA, MyHEME contains a built-in validation method to cross-confirm novel variants of interest.

S109 TP53 MUTATIONS, BUT NOT DELETION OF TP53 AND CDKN2A, HAVE INDEPENDENT PROGNOSTIC VALUE IN MANTLE CELL LYPHOMA TREATED BY THE NORDIC (MCL2 AND MCL3) REGIMEN

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Background: During the past decades, the outcome of MCL treatment has improved substantially in younger patients. However, the course of disease remains heterogeneous, and there is a need for better stratification of patients with poor responses from those with durable responses. The Nordic trials, MCL2 and MCL3, represent standard-of-care regimens for younger MCL patients.

Aims: Preliminary analyses of diagnostic samples from MCL2 and MCL3, show that TP53 mutations are associated with significantly poorer outcome. Recently, deletions of TP53 and CDKN2A was shown to confer negative impact in a cohort similar to the Nordic.(Delfau-Larue et al., 2015) Thus, in this study we aim to describe the prevalence and impact of deletions of TP53 and CDKN2A in the light of TP53 mutations.

Methods: Fresh frozen DNA from diagnostic bone marrow samples from MCL2 and MCL3 were analyzed. In both trials, patients received intensified first-line induction therapy with alternating courses of R-CHOP and R-hd-Cytarabine and consolidation with high-dose therapy and ASCT. (Geisler et al., 2008; Kolstad et al., 2014). Targeted NGS of ATM, CCND1, TP53, KMT2D, NOTCH1, NOTCH2, WHSC1 and BIRC3 was performed by Ion Torrent Technology. Cut-off for calling a mutation was set to a variant allele frequency >3%. Median coverage was >2700X. Copy Number Variations (CNVs) of TP53 and CDKN2A were measured by droplet digital PCR by commercially available assays, and RPP30 used as a standard control.

Results: We investigated the presence of CDKN2A and TP53 deletions in diagnostic samples from 175 and 157 patients, respectively. Patients were treated <6 years (median 58, range 37-65). Fifty-three percent were either MIPI intermediate- or high-risk, 17% had blastoid morphology and 42% had KI67>30%, and 83% had bone marrow involvement at diagnosis. After a median follow-up of 9.2 years, median overall (OS), progression-free survival (PFS) and cumulated incidence of relapse (CIR) of all patients were 12.4, 10.2 and 10.2 years, respectively. In our mutational analyses (n=147), only TP53 had prognostic impact in multivariate analyses (MVA). Outcome of the 15 patients (10%) with TP53-mutations was poor with a median OS, PFS and CIR of 1.8, 1.0 and 1.2 years (p=0.0001 for all three outcomes), respectively. Preliminary analysis of TP53 deletions in 28 patients (16%) showed that del-TP53 was associated with mutations of TP53, MIPI high risk, blastoid morphology and KI67>30%. Del-TP53 was associated with KI67>30%, but no other high risk markers. Altogether, 31 (25%) of 122 patients harbored a deletion and/or mutation in TP53 and 4 (3%) carried both aberrations. In univariate analyses, del-TP53 was significantly associated with poor OS (p=0.01), but not PFS and CIR, whereas del-CDKN2A was significant for CIR (p=0.02), but not OS and PFS. Patients with both deletions did significantly worse for all three endpoints. In MVA, (including all factors with significance in univariate analyses: MIPI, blastoid morphology and KI67-index>30%), del-TP53 mutations, del-TP53 and del-CDKN2A) only mutations of TP53 remained a significant predictor of outcome.

Summary/Conclusions: Here we evaluate the impact of TP53- and CDKN2A-deletions in the context of TP53 mutations of younger, optimally treated MCL patients. In line with previous reports, both deletions were associated with poor outcome; however, in multivariate analyses only TP53 mutations was an independent prognostic factor, substantiating its role as a biomarker for response to the standard-of-care immune-chemotherapy.
MRD directed treatment in AML

DEEP MOLECULAR RESPONSE TO GILTERITINIB IMPROVES SURVIVAL IN FLT3 MUTATION-POSITIVE RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA

To assess molecular response to gilteritinib in a CHRYSALIS subpopulation.

Methods: This exploratory analysis evaluated molecular response in patients aged ≥18 years with FLT3-ITDmut+R/R AML who had been treated with 120 or 200 mg/d gilteritinib. FLT3-ITDmut+ patients who had received gilteritinib 120 or 200 mg/d, 80 patients had bone marrow aspirates obtained at baseline and at ≥1 additional time point. FLT3-ITD and total FLT3 were quantified by next-generation sequencing to assess molecular response. A Cox regression model of OS by Kaplan-Meier estimation established a FLT3-ITD:total FLT3 ratio (ITD signal ratio) of 10−2 as the threshold for improved survival.

Results: Of the 147 FLT3-ITDmut+ patients who had received gilteritinib 120 or 200 mg/d, 80 patients had bone marrow aspirates at baseline and at ≥1 additional time point, and were included in this analysis. The composite response rate (defined as CR plus CRi plus PR) for these 80 patients was 55%. During response, 20 patients (25%) had an ITD signal ratio of ≤10−2. Of these 20 patients, 18 had an ITD signal ratio of ≤10−3 (major molecular response [MMR]) and 13 had an ITD signal ratio of ≤10−4 (minimal residual disease [MRD] negative). The median time to achieve minimum ITD signal ratio was 54 days. Elimination of morphologic leukemia was observed in 80% of patients with ITD signal ratios <10−2. Patients who had an ITD signal ratio <10−2, MMR, or were MRD negative had significantly longer median OS than those who did not (Table 1 and Figure 1).

Table 1. Overall survival in subjects who achieved a molecular response compared with those who did not by depth of response.

<table>
<thead>
<tr>
<th>Molecular Response</th>
<th>Achieved a Molecular Response</th>
<th>Did not Achieve a Molecular Response</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITD signal ratio ≥10−2</td>
<td>20 (241–248-NA)</td>
<td>80 (99–142–234)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ITD signal ratio &lt;10−2</td>
<td>15 (417–228-NA)</td>
<td>67 (231–344–246)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Figure 1.

Summary/Conclusions: Molecular responses to gilteritinib in FLT3-ITDmut+R/R AML correlated with improved OS. This is the first demonstration of a robust molecular response to a FLT3 inhibitor in AML. These data suggest that the ITD signal ratio may predict a durable clinical benefit of gilteritinib therapy and validate FLT3 as a critical therapeutic target in AML.
ASCT can be avoided if MRD is not detectable; if MRD is positive, ASCT can prolong OS and DFS to equalize those of the low-risk category. ASCT was delivered to 2/3 of pts in the high-risk category, using all the available sources of stem cells.

Figure 1.

S112

**GRAFT VERSUS LEUKEMIA EFFECT OF ALLOGENEIC STEM CELL TRANSPLANTATION AND MINIMAL RESIDUAL DISEASE IN PATIENTS WITH AML IN FIRST COMPLETE REMISSION**

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**Background:** The detection of minimal residual disease (MRD) in patients with acute myeloid leukemia (AML) may improve future risk-adapted strategies of AML treatment. The presence of MRD before induction treatment has firmly been shown to predict for relapse and overall outcome, irrespective of type of post-remission treatment (PRT). Currently it is unknown whether and how the presence or absence of MRD should guide the application of allogeneic hematopoietic stem cell transplantation (alloHSCT) as PRT.

**Aims:** We addressed whether and to what extent alloHSCT quantitatively reduces relapse as compared to conventional post-remission treatment (PRT) in upfront treated patients with MRD positive or MRD negative AML in first hematological complete remission (CR1).

**Methods:** A total of 1,511 patients were treated in subsequent HOVON-SAKK AML trials of whom 547 patients obtained a CR1, received PRT and had available flow cytometric MRD prior to PRT. MRD positivity was defined by more than 0.1% cells with a leukemia associated phenotype within the white blood cell compartment. MRD status was not known by clinicians during AML treatment. PRT consisted of alloHSCT (n=228), or conventional PRT as a probional third cycle of chemotherapy (n=160) or autologous HSCT (n=105). Endpoints of the study included overall survival (OS), relapse-free survival (RFS), and cumulative incidences of relapse and non-relapse mortality (NRM) at 4 years. A time-dependent analysis was performed by applying multivariable Cox regression with time-dependent covariate alloHSCT with the cumulative incidence of relapse as primary endpoint.

**Results:** MRD was positive in 120 (24%) patients after induction chemotherapy before proceeding to PRT. The latest European LeukemiaNet risk classification was similarly distributed among MRD negative and MRD positive patients. No differences were present in transplant characteristics in MRD positive and MRD negative patients. OS and RFS was significantly better in patients without MRD prior to PRT as compared to MRD positive patients (56±2% compared to 50±5% at 4 years, p<0.002, and 58±3% compared to 38±4%, p<0.001, respectively). Improved outcome was mainly caused by a lower cumulative incidence of relapse in MRD negative patients as compared to MRD positive patients (32±2% compared to 54±4% at 4 years, p<0.001, respectively), while NRM was not significantly different and estimated 10±1%. NRM split by EBMT risk assessment and that it especially enables to identify very poor risk patients in

**Summary/Conclusions:** The graft-versus-leukemia effect of alloHSCT is equally present in MRD positive and MRD negative patients, which advocates a personalized application of alloHSCT taking the risk of relapse determined by AML risk group and MRD status as well as the counterbalancing risk of NRM into account.

S113

**LEUKEMIC STEM CELL FREQUENCY COMBINED WITH MRD IS AN IMPORTANT BIOMARKER TO PREDICT RELAPSE IN ACUTE MYELOID LEUKEMIA: RESULTS FROM A PROSPECTIVE H102 STUDY**

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**Background:** Despite up-to-date risk algorithms, outcome in acute myeloid leukemia patients is still difficult to predict. Even in good risk patients relapses occur. Further refinement of currently used risk classifications is therefore warranted. Measurable residual disease (MRD) is a well-known risk factor and the independent prognostic impact of MRD was shown for patients independent on risk groups. Nowadays prospective studies are designed on which therapy is adapted based on MRD-positivity or negativity. Although this is a major improvement for risk stratification, relapses occur in a substantial proportion of MRD-negative patients. Previous retrospective studies have shown that the leukemic stem cell (LSC) frequency harbors important prognostic information as well (Bradbury et al., Leukemia 2015), even within MRD-negative patients (Terwijn et al. Plos one, 2013).

**Aims:** In this study we used data of the HOVON/SKAK H102 trial to prospectively define, using flow cytometry, the leukemic CD34+CD38- stem cell frequency and MRD frequencies to investigate impact on patient outcome.

**Methods:** In 242 patients who achieved morphologic complete remission, both LSC and MRD data after two cycles of chemotherapy treatment were available. MRD-positivity was defined as a percentage of MRD-positivity of about 1% (as compared to total amount of WBCs) and LSC-positivity was defined as a CD34+CD38-LSC percentage above 0.0000% (LSC cut-off 0.0000%; thus no LSC-positivity). Measurable residual disease (MRD) was defined based on MRD-positivity or negativity. Although this is a major improvement for risk stratification, relapses occur in a substantial proportion of MRD-negative patients. Previous retrospective studies have shown that the leukemic stem cell (LSC) frequency harbors important prognostic information as well (Bradbury et al., Leukemia 2015), even within MRD-negative patients (Terwijn et al. Plos one, 2013).

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all different currently used risk categories. These data urge to include both MRD and LSC in future AML risk classification to better inform post-remission treatment.

DEFINITION OF PARTIAL RESPONSE IN YOUNGER AML PATIENTS AFTER FIRST INDUCTION COURSE MAY BE EXTENDED BY INCLUSION OF IMMUNOPHENOTYPIC DETECTION OF MEASURABLE RESIDUAL DISEASE IN CR


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Background: In AML response by morphology after a first cycle of induction therapy is used to guide further therapy including second cycles of induction and choice of consolidation. It is still uncertain how the quality of response post cycle 1 with inclusion of MRD assessment impacts on outcomes within AML risk subgroups including NPM1 wild type standard risk and whether this adds information to MRD status in CR post cycle 2.

Aims: To quantify the effect of MRD positivity for response after each cycle of induction therapy in younger patients with AML.

Methods: As part of the UK NCRI AML17 trial (ISRCTN: 55675535) for patients with AML or high risk MDS up to the age of 60, prospective flow cytometric MRD (MFC-MRD) monitoring was performed after each course of induction. Any level of MRD detected was considered MRD+ (sensitivity thresholds: ~0.02% by tracking diagnostic leukemic aberrant phenotypes /LAIP, ~0.05-0.1% by “different-from normal” blast LAIP). Clinicians were not informed of MFC-MRD results. Following their first cycle of induction with daunorubicin/ara-C based therapy, patients were allocated a risk group by a validated score (comprising cytogenetics, WBC, age, secondary disease, blast response to cycle 1 and mutation status). Poor risk patients received intensified therapy in cycle 2 with a view of proceeding to SCT.

Results: MFC-MRD results after either induction course are available for 1555 patients randomised from 4/09-12/14 (median age 51, range 0-73). Cycle 1 (C1) response data with MFC-MRD was available for 1,400 patients. 70% achieved morphological CR at this time-point; 14% had resistant disease (RD) and 16% were in partial remission (PR) according to clinician. Of patients in CR (n=984) 56% had detectable MFC-MRD (MRD+). Excluding poor-risk patients 14% of patients did not achieve CR (7% RD, 7% PR), 51% of patients in CR were MRD+. 5 year OS for MRD- vs MRD+ was 63% vs 44% vs 37% vs 25% for all patients; 69% vs 51% vs 50% vs 30% excluding poor risk patients and 66% vs 49% vs 49% vs 30% for standard risk alone (Figure 1). The similar OS in this group between CR MRD+ and PR at C1 was maintained in NPM1/wt standard risk patients and if censored at stem cell transplant. 771 patients were in CR post cycle 2 (C2) and provided MFC-MRD data. As expected, there were significant differences in 5 year OS between CR MFC MRD+ vs CR MFC MRD- for all patients (35% vs 83%) and excluding poor-risk (38% vs 70%, n=512). Importantly post cycle 2 MFC-MRD status also differentiated OS for NPM1/wt standard risk patients with 5 year OS of 32% vs 64% (P=0.002) for MRD+ vs MRD-. In stratified analyses, there was some evidence that the effect of MRD positivity on OS was lower in poor-risk patients (test for trend p=0.02 for both C1 and C2). The effect of MFC-MRD status on relapse and OS appeared greater at C2 (relapse, OR 2.00(1.56-2.55), p<0.001; survival, OR 1.80(1.42-2.28) p<0.001) than C1 (relapse, OR 1.69(1.37-2.07), p<0.001; survival, OR 1.46(1.19-1.79) p<0.001). In patients with data for both time points, C2 MRD remained significant on OS when adjusting for C1 response. 24 patients converted from C1 MRD- to C2 MRD+, with a poor prognosis (15 relapses, 13 deaths). C1 MRD-/C2 MRD- had the best prognosis.

Summary/Conclusions: MFC-MRD in CR post cycle 1 has similar outcomes to partial remission in younger patients with AML, particularly in patients with good and standard risk disease. Assessment of MFC-MRD post cycle 2 appears to provide additional discrimination to cycle 1: MFC-MRD in courses 1-2 may be useful in further stratifying standard risk patients.
 FBXW7 MUTATIONS LEAD TO ACCUMULATION OF NOTCH1, HIF1-α AND c-MYC IN CLL CELLS

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Background: Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with recurrent mutations that are of pathogenic and prognostic relevance. Mutations in FBXW7 are among the most common mutations in CLL, yet their functional consequences are unknown. FBXW7 is an E3 ubiquitin ligase that ubiquitylates oncoproteins like NOTCH1, HIF1-α and c-MYC and thereby targets them for proteasomal degradation.

Aims: To determine the functional impact of FBXW7 mutations in CLL cell lines and primary samples.

Methods: FBXW7 mutations were induced using CRISPR/Cas9 technology in the wild-type HG3 cell line. The induced truncation of the WD40 domain of FBXW7 in the HG3 cell line resulted in the accumulation of NOTCH1 and c-MYC. In primary CLL cells, the protein levels of FBXW7 substrates were examined.

Results: FBXW7 mutations were found in 41/905 (4.5%) of CLL patients. The most common mutations of FBXW7 were missense mutations (32/41) that target the substrate binding domain of the FBXW7 protein as well as non-sense mutations (4/41). Interestingly, 5 patients harbored two concurrent driver mutations. Mutations were verified by allele-specific PCR or a second round of generation sequencing. Mutations at any CCF had prognostic value, ii) mutations with a CCF above a certain threshold impacted the outcome of the patients.

Summary/Conclusions: Mutations in FBXW7 are frequently found in CLL, especially missense and nonsense mutations affecting the WD40 domain. We hypothesize that this has functional consequences on FBXW7 substrate binding and hence to accumulation of oncogenes. The truncation of the WD40 domain of FBXW7 in the HG3 cell line resulted in the accumulation of protein substrates and corresponding increase of their activity implicated in the pathogenesis of CLL. Taken together our data show that FBXW7 can target proteins for degradation that are commonly dysregulated in CLL and that drive disease progression.

haematologica | 2017; 102(s2) | 9
Results: All 102 patients included in the cohort (51 CLL and 51 healthy donors) were genotyped for 264,124 SNP loci using the Illumina Infinium HumanOmniExpress BeadChip. We employed a range of bioinformatic tools to analyze the data in an integrative way.

Methods: We established a novel karyotyping methodology and SNP genotyping of the karyotypic breakpoints of CLL patients. Further studies included the identification of the clonal expansions in CLL cells using whole-exome sequencing and single-cell sequencing of the CLL samples.

Aims: The primary objective of this study was to provide insights into the evolutionary trajectories of CLL cells and to identify potential therapeutic targets for CLL treatment.

Results: We identified a total of 410 karyotypic breakpoints in the CLL samples, including 130 new breakpoints. The analysis revealed a spectrum of clonal expansions across the CLL samples, with the most frequent rearrangements involving chromosomes 17, 11, and 14. Whole-exome sequencing of the CLL samples revealed a high degree of genetic heterogeneity, with 114 different somatic mutations identified in the 102 patients.

Conclusion: Our study provides a comprehensive overview of the genomic landscape of CLL and identifies potential therapeutic targets for future clinical trials.

S118 THERAPEUTIC DISRUPTION OF THE BAFF-B-CELL RECEPTOR CROSS-TALK IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

C. Paiva1, T. Rowland1, B. Sreekantham1, O. Danilova1, A. Danilov1,∗
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Background: Although small molecule inhibitors of BCR-associated kinases (BCRi) revolutionized therapy in CLL, they provide incomplete responses. Tumor necrosis factor receptor superfamily ligands BAFF and APRIL induce NFκB, which in turn upregulates pro-survival Bcl-2 family proteins and thereby drives anti-apoptotic responses, potentially accounting for resistance to BCRi. The exact roles of the individual NFκB pathways, as well as the implications of targeting BCR in context of BAFF signaling in CLL remain understudied.

Aims: Our aim was to investigate the molecular mechanisms underlying the cross-talk between BCR and BAFF signaling in CLL cells.

Methods: We employed a range of bioinformatic tools to analyze the data in an integrative way.

Results: We identified a total of 410 karyotypic breakpoints in the CLL samples, including 130 new breakpoints. The analysis revealed a spectrum of clonal expansions across the CLL samples, with the most frequent rearrangements involving chromosomes 17, 11, and 14. Whole-exome sequencing of the CLL samples revealed a high degree of genetic heterogeneity, with 114 different somatic mutations identified in the 102 patients.

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Conclusion: Our study provides a comprehensive overview of the genomic landscape of CLL and identifies potential therapeutic targets for future clinical trials.
Background: MYBL2 is a transcription factor with roles in the cell cycle and genome integrity. MYBL2 is located on chromosome 20, within a region commonly deleted in human blood disorders (del20q). Our published data shows that reduced levels of MYBL2 predispose to development of myelodysplastic syndromes (MDS)-like disease in mouse models during ageing, indicating that MYBL2 could be acting as a tumour suppressor gene within del20q abnormality. Moreover, our previous work demonstrated that regardless of del20q deletion, MYBL2 expression is reduced in CD34+ bone marrow cells from MDS patients with worse prognosis. Because it has been shown that the cell of origin of MDS is the haematopoietic stem cell (HSC) and given the role of MYBL2 in DNA replication fork progression and maintenance of genome integrity, we hypothesised that low MYBL2 levels in HSC could contribute to elevated somatic mutations through changes in DNA repair pathways and drive disease development.

Aims: The aim of this study was to determine if low MYBL2 levels affect the double strand break (DSB) DNA repair damage response in HSC.

Methods: In this study we used our mouse model in which animals express ~50% normal levels of MYBL2 (Mybl2+/∆). We characterised the ability of HSCs from young (7 weeks) and old (70 weeks) animals to respond to in vivo ionising radiation (2Gy) in terms of proliferation, apoptosis and colony forming ability. We measured the activation of the two main DNA repair pathways operating in the cells to deal with DSB: the error prone non-homologous-end-joining (NHEJ) and the error-free homologous recombination (HR) by assessing 53BP1 and Rad51 recruitment by immunofluorescence, respectively. Finally, we analysed the frequency of chromosome abnormalities present in the progeny of Mybl2+/∆ HSC that have previously been irradiated to determine the long term effects of changes in DNA repair.

Results: We observed that Mybl2+/∆ HSCs had limited proliferative potential and displayed an increased sensitivity to ionizing radiation which increased during ageing. Mybl2+/∆ HSCs also displayed altered kinetics of 53BP1 and Rad51 recruitment and clearance, including retention of 53BP1 foci at later time points following irradiation and decreased levels of Rad51 foci when compared to Mybl2+/- HSCs. Using plasmid functional assays, we showed that Myb2+/∆ HSCs repair quite efficiently by NHEJ, but this efficiency is disrupted when cells are challenged with ionising radiation. Furthermore, Myb2+/∆ HSCs have increased sensitivity to inhibition of DNA-PKc (required for NHEJ) but not ATM (required by HR). We also observed that after ionizing irradiation Myb2+/∆ HSC progeny displayed an increased percentage of chromatids with fragile telomeres. Moreover, by making use of publically available RNA-seq data from Cimino et al, we have identified a clear association between low MYBL2 levels and low expression of DNA-repair genes in patients with worse prognosis.

Summary/Conclusions: In summary, we have shown that decreased expression of MYBL2 leads to an imbalance in the DSB DNA-repair pathway choice, ultimately resulting in increased genomic instability of the blood cell progeny. These findings are supported by a signature of deregulated DNA-repair genes which strongly associates with low MYBL2 levels in MDS patient samples, providing a mechanistic understanding for the progression of blood disorders occurring during ageing. This study demonstrates a role for MYBL2 in DSB repair in HSCs and suggests that low levels of MYBL2 in human MDS could contribute to the emergence of further genetic abnormalities by deregulation of DNA-repair pathways.
THE VALUE OF NGS PANEL SEQUENCING TO MOLECULARLY DEFINE MEYLOID MALIGNANCIES AND CLARIFY BORDERLINE CASES: A STUDY ON 39 GENES IN 1143 PATIENTS
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Background: The 2016 revision of the WHO classification for myeloid malignancies includes numerous molecular markers for classification and prognostication. Next generation sequencing allows analyzing relevant genes in one panel.

Aims: To exploit clinical usefulness of panel sequencing in routine diagnostics in order to describe genetic changes and use respective patterns in cases with undetermined morphology.

Methods: According to WHO 2016, 1143 patients were morphologically categorized as AML (n=261), MDS (n=176), MPN (n=19), CMML (n=51) or AML/MDS (n=21) and MDS/MPN overlap (n=28). Patients, who did not fulfill all characteristic criteria or had insufficient/unsatisfactory quality, were classified as possible AML (n=28), MDS (n=211), MPN (n=38), CMML (n=14) and as reactive (n=193) or unclear (n=136). DNA was isolated from BM (n=958) or PB (n=185) for NextSeq or MiSeq sequencing after TruSeq library preparation (all Illumina, San Diego, CA). Data was analyzed with SeqNext 4.3 (JSI Medical Systems, Kippenheim, Germany).

Results: Analyzing 39 genes, we found ≥1 molecular change in 90% of patients (500/556) with a defined morphologic diagnosis (median: 2 genes; max: 7). In de novo AML, 212/229 (93%) patients showed ≥1 molecular hit, of which 211 (92%) had aberrations that define WHO categories or have prognostic (according to AML FAB classification validative variables). More than 50% of cases were found in 166/229 patients (72%), including information of adverse impact (e.g. of 68 NPM1 positive patients, 17 had DNMT3A mutations and 20 FLT3-ITD). Following NPM1, RUNX1 was the second most frequently mutated gene (46/225; 20%) and mutations were significantly more common in patients with ≥3 aberrations (37/77; 48%; 6/141, 4%; p<0.01). A similar RUNX1 pattern was found in s-AML and t-AML. In the cohort of “possible AML” (including MDS overlap and diagnostics), 45/48 (94%) patients had ≥1 hit. Most frequently mutated were ASXL1 (16/48; 33%), TET2 (32%; 14/44) and SRSF2 (29%; 14/48); 16% had all three mutated. This combination is also the most frequently three-way interaction in CMML (10/44; 23%). In MDS, 124/157 (79%) cases showed mutations, of which 108 had ≥3 prognostic change (according to Bejar, 2015). The prognostically favorable SF3B1 mutation was present in 31/157 (20%) and significantly enriched among cases with ring sideroblasts (p=0.001). Overall, TET2 showed the highest mutation rate (25%) and was also the most commonly mutated gene in cases with ≥3 molecular markers for clonal disease in 47% (91/199), 36% (43/118) or 17% (36/211) of cases, respectively (excluding sole ASXL1, DNMT3A, TET2 mutations with <10% burden).

Summary/Conclusions: WHO 2016 requires information on numerous genes for diagnosis, prognosis and therapeutic decisions. This challenges conventional approaches, because these genes are present at least one molecular marker for clonal disease in 47% (91/199), 36% (43/118) or 17% (36/211) of cases, respectively (excluding sole ASXL1, DNMT3A, TET2 mutations with <10% burden).
Methods: We performed transcriptome sequencing of bone marrow mononuclear cells (BMMNCs) and/or CD34+ cells obtained from patients with myelodysplasia. Consensus clustering was used to identify stable patient clusters. A classifier of the gene expression-based subgroups was constructed using the 100 CD34+ cell samples as a training set, followed by validation in an independent cohort of 183 MDS patients. Another classifier was constructed using BMMNC samples from 51 patients, who had been assigned to the subgroups by the gene expression data of their CD34+ cells. Prognostic significance of the model was tested in 114 patients of myelodysplasia.

Results: Unsupervised clustering of gene expression data of bone marrow CD34+ cells from 100 patients identified two subgroups (Class-I and Class-II). The patients in the Class-II subgroup had higher percentages of bone marrow blasts compared to those in the Class-I subgroup (median 2% vs 11%, \( P < 0.01 \)). Pathway analysis revealed up-regulation of many signaling pathways in the Class-II subgroup. The Class-I subtype showed highly significant up-regulation of the genes related to erythroid lineages. The erythroid signature was rather suppressed in the Class-II subtype, which was characterized by increased expression of genes related to progenitor cells. Compared to the Class-I subtype, the Class-II subtype was associated with a significantly shorter survival in both univariate (hazard ratio [HR] 5.0 [95% CI, 1.8–14], \( P < 0.001 \)) and multivariate analysis (HR 6.8 [95% CI, 1.5–32], \( P = 0.015 \)). High frequency of leukemic transformation in the Class-II subgroup (38%) contrasted to no leukemic transformation in the Class-I subgroup. The prognostic significance of our classification was validated in an independent cohort of 183 patients. We also constructed a model to predict the subgroups using gene expression profiles of BMMNCs. The model was applied to 114 patients with BMMNC samples, of whom 47 (41%) were predicted to be the Class-I subgroup. Compared to the predicted Class-I subgroup, the Class-II subgroup was associated with a significantly shorter survival in univariate analysis (HR 7.2 [95% CI, 3.0–17], \( P < 0.001 \)). Again, association was more pronounced for leukemic transformation (HR 18 [95% CI, 4.2–80], \( P < 0.001 \)) than for overall survival. Multivariate analysis also demonstrated that the predicted Class-II subgroup was independently associated with leukemic transformation (HR 7.3 [95% CI, 1.3–41], \( P = 0.024 \)). Finally, we compared the prognostic value of our model with that of the LSC17 score, which has recently been proposed to predict a subset of poor risk acute myeloid leukemia based on the expression of 17 genes related to a leukemic stem cell signature. Our model outperformed the LSC17 score in predicting clinical outcomes of myelodysplasia, especially leukemia progression. The Class-II signature was shown to be more dramatically up-regulated during clonal evolution of myelodysplasia than the LSC17 score, which might be the basis of a better prediction of leukemia progression in our model.

Summary/Conclusions: Comprehensive transcriptomic analysis identified two subgroups of myelodysplasia with biological and clinical relevance, which could improve risk prediction and treatment stratification of myelodysplasia.

Lymphoma biology

S124

GENETIC ALTERATIONS INVOLVING PROGRAMMED DEATH LIGANDS IN EPSTEIN-BARR VIRUS-ASSOCIATED LYMPHOMAS


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Background: Checkpoint blockade using anti-PD-1/PD-L1 antibodies is a highly promising therapy for cancer, frequently showing dramatic anti-tumor responses in a wide variety of tumor types. Particularly, an exceptional response to anti-PD-1 antibodies has been demonstrated for classical Hodgkin lymphoma (HL), which is characterized by frequent copy number gains/amplifications in PD-L1 and/or PD-L2, suggesting a close association between these genetic alterations and the therapeutic response to these agents. Recently, we have reported frequent structural variations (SVs) in adult T-cell leukemia/lymphoma (ATL) caused by human T-cell leukemia virus type-1 (HTLV-1). These SVs invariably affect 3’-untranslated region (UTR) of PD-L1, leading to promi- nently increased expression of PD-L1, thus promoting immune escape of virally infected cells. We hypothesized that a deregulated PD-1/PD-L1 axis might play a critical role in evasion from anti-viral immunity before these cells are clonally selected for neoplastic proliferation.

Aims: Epstein-Barr virus is a DNA tumor virus closely associated with various human cancers, including B- and natural killer (NK)/T-cell lymphomas, in which genetic alterations involving PD-L1/PD-L2 may also be relevant to cancer evolution. In this study, to assess this hypothesis, we interrogated a variety of lymphomas for genetic abnormalities affecting PD-L1 and PD-L2, especially focusing on EBV-associated lymphomas.

Methods: SVs and other genetic lesions affecting PD-L1 and PD-L2 were analyzed by targeted-capture sequencing with cRNA baits designed for capturing the entire sequences of PD-L1 and PD-L2 genes, including exons, introns, and 5′- and 3′-UTRs. More than 400 samples were analyzed obtained from different subtypes of non-Hodgkin lymphomas, including EBV-associated lymphomas, such as EBV-positive diffuse large B-cell lymphoma (DLBCL) and NK/T-cell malignancies.

Results: SVs and/or focal copy number gains involving PD-L1 genes were successfully detected in various B-cell and T/NK-cell lymphomas, albeit at generally low frequencies (<10%). These lesions were the most frequently observed in PMBL and accounting for more than 60% of the cases. Of note, high frequency (17−57%) of PD-L1/PD-L2-involved abnormalities were observed in mature NK/T-cell neoplasms, including extranodal NK/T-cell lymphoma, aggressive NK cell leukemia, and EBV-positive T-cell lymphoproliferative disorder, all of which were positive for EBV. Moreover, a substantial proportion (22%) of EBV-positive DLBCL cases possessed these lesions, whereas EBV-negative cases rarely exhibited these alterations (2%, \( P < 0.01 \)). For both PD-L1 and PD-L2 SVs, despite a large diversity of SV type (deletions, inversions, tandem duplications, and translocations), most of SVs resulted in a 3′-UTR truncation, while the replacement of PD-L1 or PD-L2 promoter with an ectopic regulatory element was rarely observed. Interestingly, PD-L1 SVs were detected in both B- and T-cell lymphomas, whereas PD-L2 SVs were found exclusively in B-cell lymphomas.

Summary/Conclusions: We delineate the entire picture of genetic alterations involving PD-L1 and PD-L2, the close association between these genetic alterations and EBV-associated lymphomas. Our finding help to understand their pathogenesis and develop a new diagnostic strategy to identify patients who potentially benefit from PD-1/PD-1 blockade therapy in non-Hodgkin lymphomas.

S125

FOXO1 CONTROL CD20 EXPRESSION AND INFLUENCE B-CELL LYMPHOMA RESPONSE TO RITUXIMAB-BASED IMMUNOTHERAPY

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Background: Recurrent somatic mutations of N-terminal region of FOXO1,
shown previously to increase FOXP1 nuclear localization and activity, have been linked to diminished survival in DLBCL patients uniformly treated with rituximab-based immunotherapy. Although the contribution of FOXP1 mutations to the therapeutic resistance of B-NHLs becomes apparent, the molecular mechanism underlying this phenomenon has not been explained so far. The diminished levels of CD20 on the cell surface of tumor cells are among several potential mechanisms underlying the resistance to treatment with anti-CD20 monoclonal antibodies.

Aims: We have recently reported that the tonic BCR signaling activates FOXP1, and that inhibitors of the downstream BCR signaling pathways down-regulate CD20 expression. Therefore, here we sought to determine whether FOXP1 might regulate the abundance of CD20 on the surface of tumor cells thus influencing the response to rituximab-based therapies.

Methods: We used CRISPR/Cas9 genome editing technology and lentiviral transduction to study the role of FOXP1 protein in CD20 regulation. qRT-PCR and Dual Luciferase Assays was done to determine the influence of FOXP1 on CD20 transcription. To decipher the molecular interaction between FOXP1 and CD20 promoter we performed EMSA and Chip experiments. For animal studies we used SCID Fox Chase mice model. All in vitro experiments were carried out at the animal facility of The Francis Crick Institute in accordance with the guidelines and were approved by the Ethics Committee.

Results: To determine the potential role of FOXP1 protein in CD20 regulation, we disrupted FOXP1 focus using the CRISPR/Cas9 genome editing technology in Raji cells. In in vitro complement-dependent cytotoxicity assay we show that ablation of FOXP1 results in upregulation of CD20 levels and improved sensitization to rituximab efficacy. To see whether FOXP1-dependent up-regulation of CD20 translates into tumor growth, we performed xenografts experiments. In vivo experiments were complemented by xenografts models of DLBCL. We found that mice treated with systemic rituximab survived longer when inoculated with sgFOXO1-transduced Raji cells as compared with mice inoculated with control Raji cells. Consistently, using clinically tested PI3K-AKT inhibitors - MK-2206 and GDC-0068 – in a set of CLL primary samples we show that also pharmaceutical inhibition of FOXO1 activity upregulated surface CD20 levels. Moreover, we demonstrated that FOXP1 regulates the CD20 promoter activity. In different B-cell lymphoma cell lines MK-2206 and GDC-0068 significantly downregulated the levels of MS4A1 transcript (encoding CD20). Finally, using both EMSA and Chip assays we detected specific binding of FOXP1 to the MS4A1 promoter to the extent comparable to other known FOXP1 target genes.

Summary/Conclusions: Collectively, our results indicate that FOXP1 is a strong negative regulator of CD20 expression and add new insights into the mechanisms underlying the contribution of FOXP1 mutations to the resistance of B-NHLs to B-CHOP therapy. In light of current knowledge and our observations presented in this study, FOXP1 inhibition represents a novel strategy to increase the efficacy of anti-CD20 monoclonal antibodies.

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Background: ALCL is a high grade lymphoma characterized by anaplastic morphology, expression of CD30 (Ki-1) and T- or null cell phenotype. In 60% of systemic ALCL, the translocation t(2;5)(p23;q35) leads to expression of the oncogenic NPM-ALK enzyme. NPM-ALK interaction partner of ALK (NIPA) is an F-box Protein contributing to the timing of mitotic entry by defining an oscillating ubiquitin E3 ligase. NIPA deficient mice are viable but sterile due to impaired DNA double strand break repair. Co-expressed with NPM-ALK, NIPA is constitutively phosphorylated. However, the role of NIPA in NPM-ALK induced lymphomagenesis and the functional impact of this interaction remain unknown.

Aims: In this study, we aim to investigate the effect of NIPA deficiency on NPM-ALK driven cell proliferation and transformation in order to characterize the function of the protein in ALCL-induced lymphomagenesis.

Methods: Primary Nipa-/-MEFs infected with NPM-ALK were plated in soft agar to assess their transformation ability. Moreover, NIPA was downregulated through targeted genetic approaches in Karpas299 and NPM-ALK infected BaF3 cells, which were analyzed regarding proliferation, signaling, and apoptosis. To assess the impact of NIPA deletion in vivo, we used a retroviral bone marrow transplantation model resembling human ALCL. Based on a Cre/loxP system under the LCK-Promotor, NPM-ALK expression and Nipa-deletion are restricted to early T cells. In wildtype background, mice die of systemic lymphadenopathy, and bone marrow infiltration. Immunphenotyping showed a crucial role of NIPA in NPM-ALK driven lymphomagenesis. To investigate the oscillating ubiquitin E3 ligase. NIPA deficient mice are viable but sterile due to impaired DNA double strand break repair. Co-expressed with NPM-ALK, NIPA is constitutively phosphorylated. However, the role of NIPA in NPM-ALK induced lymphomagenesis and the functional impact of this interaction remain unknown.

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Summary/Conclusions: as cell cycle distribution seems not to be altered in knockout cells. Further analyses may thus elucidate NIPA as a novel molecular target for therapeutic intervention.

S128

TIGET-IV/BTHAL TRIAL OF AUTOLOGOUS HEMATOPOIETIC STEM CELLS GENETICALLY MODIFIED WITH GLOBE LENTIVIRAL VECTORS

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Background: Gene therapy for transfusion dependent beta-thalassemia, as an alternative cure to allogeneic HSCT, is based on the autologous transplantation of hematopoietic stem cells (HSCs) engineered by lentiviral vectors expressing a transcriptionally regulated human beta-globin gene.

Aims: Our contribution to this field was devoted to the clinical development of a gene therapy protocol based on high-titer vector GLOBE, use of lenogastim and plerixafor as source of HSCs and a conditioning regimen based on myeloablative treosulfan and thiotepa favoring efficient engraftment of corrected cells with reduced toxicity (TIGET-BTHAL; Eudract CT number 2014-004860-39).

Methods: On the basis of extensive efficacy and safety preclinical studies, the clinical trial TIGET-BTHAL was approved and started in 2015 at Scientific Institute San Raffaele, Milan, Italy. The clinical study foresees treatment of 10 patients: 3 adults followed by 7 minors, with a staggered enrolment strategy based on evaluation of safety and preliminary efficacy in adult patients by an independent data safety monitoring board before inclusion of pediatric subjects. The chosen route of administration of gene modified HSCs is intraosseous in the posterior-superior iliac crests, bilaterally, with the aim of enhancing engraftment and minimizing first-pass intravenous filter.

Results: As of February 2017, seven patients (3 adults and 4 pediatric patients) with different genotypes (β0/β0, β+/- and β+/-) have been treated with GLOBE-transduced CD34\textsuperscript{+} cells at a dose of 16x10\textsuperscript{6}-19.5x10\textsuperscript{6} cells/kg and a vector copy number (VCN)/cell ranging from 0.7 to 1.5. The procedure was well tolerated by all patients, with no product-related adverse events. Multilineage engraftment of gene-marked cells was observed in all tested peripheral blood and bone marrow samples. Polyclonal vector integrations profiles have been detected in the first 3 patients tested.

Summary/Conclusions: So far, the clinical outcome indicates reduction in transfusion requirement in adult patients and greater clinical benefit in younger patients. Follow up analysis are ongoing and updated clinical outcome will be presented.
to first dose, confirmed over 6 months) or NTD (<4 RBC U/8 weeks prior to first dose with baseline Hgb <10 g/dL). Pts were treated every 3 weeks subsequently for up to 5 doses; 8 cohorts were treated at dose levels from 0.2-1.25 mg/kg. Pts in the expansion cohort and those who rolled over to the ext study were treated at 0.8 mg/kg with titration up to 1.25 mg/kg (base completion NCT01745940; ext ongoing NCT02286409).

Results: A total of 64 pts were analyzed in this study (31 TD and 33 NTD), and of those, 51 enrolled in the ext study (24 TD, 27 NTD). Median (range) age (yr) was 38.5 (20-62); 67% had prior splenectomy. For TD pts, at baseline, median (range) transfusion burden was 8 U/12 weeks (4-18 U); median (SD) liver iron concentration (LiC, mg/g dw) was 5.0 (5.3). For NTD pts, at baseline, median (SD) transfusion burden was 8 LiC (5.6 ± 3.4) and calcium (Ca, mmol/L) was 2.3 (2.4). BMD was much higher compared to placebo (-0.22±5.40% vs 0.549±0.098, p=0.004) and a significant decrease in their WR BMD (0.520±0.099 g/cm2 vs 0.549±0.099, p=0.008). The percentage increase of L1-L4 BMD was higher in DMB arm than in placebo arm (6.02±5.30% vs 3.11±5.46%, respectively; p=0.03), while the advantage of DMB regarding WR BMD was much higher compared to placebo (-0.22±5.40% vs -4.15±8.58%, respectively; p=0.02) as well as in FN BMD (p<0.001). No grade 3 or 4 toxicity was observed in all of the 64 pts.

Summary/Conclusions: This first analysis of our phase 2b study regarding the effects of DMB on BMD of different sites (the results of bone markers will be presented in the conference), suggests that DMB, given twice per year, increases the BMD of the L1-L4 more efficiently than placebo (in combination with RBC transfusion). Long-term studies in TM patients are needed to confirm the efficacy of DMB in the prevention of osteoporosis, with excellent safety profile. Furthermore, DMB increased the FN BMD, which was not increased in the placebo arm, while DMB has also a positive effect on WR BMD compared to placebo. These data support the use of DMB for the management of TM-induced osteoporosis.

S131
LONG-TERM HEALTH STATUS AFTER HSC TRANSPLANTATION FOR THALASSEMAIA: THE FRENCH EXPERIENCE
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Background: In clinical practice, allogeneic hematopoietic stem cell transplantation (HSC-T) is the only treatment offering a definitive cure for patients with beta-thalassemia. Its outcome has improved over the last 3 decades with the advent of highly effective conditioning regimens. This report provides long-term follow-up data for a cohort of 134 transplanted patients from 8 French centers. Patients and Methods: This French retrospective study included patients who successfully received allogeneic HSC-T between 1985-2012 and were alive at least 2 years after HSC-T. Study was based on data collected in the national registry of patients with beta-thalassemia and conducted in collaboration with the French Society of Hematology. Data were recorded by physicians through reference or transplant center visits. Collected data included medical examination results, long-term treatments administered and laboratory tests (serum ferritin, Hb, liver enzymes, creatinine level and thyroid evaluation). Linear mixed model was used to analyze data evolution over time (for height and weight SDS, SF, HB values).

Results: A total of 134 patients had received allogeneic HSC-T for beta-thalassemia in France from 1985 to 2012. 107/134 patients experienced successful HSC-T (6 after a second transplant) and were alive 2 years after transplantation. Six were not analyzed (back to their country or lost of follow-up) and two died of chronic respiratory failure related to transplant at day 67 and 132 respectively. 19% of the patients were on regular transfusions. Median age at HSC-T was 5.9 years (8 months-26 years). The source was bone marrow in 85% of cases and a matched sibling donor was used in 90% of cases. Conditioning mostly consisted (85%) of busulfan and cyclophosphamide (oral busulfan in 52%). Median age at the last visit was 19 years. Chronic complications for 18% of patients delayed or compromised engraftment. 8% of patients required second transplant. 1% developed grade 3 or 4 adverse events. 11% had graft vs host disease. 15% had significant infections and 5% underwent second transplant. 1% developed non-Hodgkin lymphoma. The most common complication was chronic lung disease in 19% of patients. 88% of patients had growth retardation. 93% had anemia requiring RBC transfusions. 6% of patients had allergic disorders.

Conclusions: Allogeneic HSCT for beta-thalassemia major is a safe and effective treatment option with a disease free survival rate of 90% when transplant is performed in childhood from an HLA-identical sibling. Few data are available on long-term toxicity and frequency of chronic complications after transplant.

Aims: The purpose of this study was to evaluate the long-term health status after a successful allogeneic HSC-T for beta-thalassemia major in a national cohort of patients.

Methods: This French retrospective study included patients who successfully received allogeneic HSCT between 1985-2012 and were alive at least 2 years after HSC-T. Study was based on data collected in the national registry of patients with beta-thalassemia and conducted in collaboration with the French Society of Hematology. Data were recorded by physicians through reference or transplant center visits. Collected data included medical examination results, long-term treatments administered and laboratory tests (serum ferritin, Hb, liver enzymes, creatinine level and thyroid evaluation). Linear mixed model was used to analyze data evolution over time (for height and weight SDS, SF, HB values).
Background: Donor-derived red blood cells (RBC) are the most common form of cellular therapy. However, the source of cells is dependent on donor availability with a potential risk of allo-immunization and blood borne diseases.

Aims: We aim to produce unlimited numbers of cultured RBC with a defined ‘universal donor’ phenotype for transfusion purposes.

Methods: To this end we prepare for a clinical test using autologous cultured RBC to test their in vivo stability. In parallel we develop methods for unlimited production of cultured RBC. An immortal source to produce in vitro cultured RBCs (iRBC), such as iPSCs would allow selection of ‘universal donor’ RBC, or provide an autologous end product with the absence of immune reactions.

Results: The in vitro production of RBC has proven to be successful, however there are barriers to overcome prior to clinical application. e.g. xeno-free culturing methods, scale up cultures to obtain transfusion units (1-2*10^12 erythrocytes), and for iPSC we need virus- and transgene-free reprogramming protocols. To solve the above mentioned issues a customized humanized GMP-grade medium (Cellquin) was generated in order to control erythroid culture parameters and to reduce culture costs. This medium allowed 1*10^8 times erythroid expansion from PBMCs to pure adult EBL cultures within 25 days, comparable to non-GMP commercial media. To generate iPSC, a non-integrative polyclonistic episcopic vector containing (OCT4-SOX2-KLF4-cMYC-LIN28) was used to reprogram PBMC-expanded EBLs to iPSC, displaying pluripotency potential and normal karyotype. iPSCs were adapted to single cell passage allowing directed colony differentiation using a feeder-free monolayer approach. From day 6 of differentiation Cellquin was applied with lineage-specific growth factors, resulted iPSC differentiation to EBLs, and for iPSC we need virus- and transgene-free reprogramming protocols. To solve the above mentioned issues a customized humanized GMP-grade medium (Cellquin) was generated in order to control erythroid culture parameters and to reduce culture costs. This medium allowed 1*10^8 times erythroid expansion from PBMCs to pure adult EBL cultures within 25 days, comparable to non-GMP commercial media. To generate iPSC, a non-integrative polyclonistic episcopic vector containing (OCT4-SOX2-KLF4-cMYC-LIN28) was used to reprogram PBMC-expanded EBLs to iPSC, displaying pluripotency potential and normal karyotype. iPSCs were adapted to single cell passage allowing directed colony differentiation using a feeder-free monolayer approach. From day 6 of differentiation Cellquin was applied with lineage-specific growth factors, resulted iPSC differentiation to EBLs which was initiated by the appearance of p53, leading to the accumulation of mutations.

Summary/Conclusions: We showed that our monolayer approach is simple, highly controlled and compatible with upscaling. Avoiding virus-, integrative reprogramming, feeders and with our GMP-grade media we maintained a cost effective system moving toward clinical application.

S132

CD34+AND HUMAN INDUCED PLURIPOTENT STEM CELL DIFFERENTIATION TO TRANSFUSION READY RED BLOOD CELLS

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Background: From day 6 of differentiation Cellquin was applied with lineage-specific growth factors, resulted iPSC differentiation to EBLs which was initiated by the appearance of p53, leading to the accumulation of mutations.

Summary/Conclusions: We showed that our monolayer approach is simple, highly controlled and compatible with upscaling. Avoiding virus-, integrative reprogramming, feeders and with our GMP-grade media we maintained a cost effective system moving toward clinical application.

S133

FUNCTIONAL PROTEOMICS IDENTIFIES SETD2 AS A CRITICAL EFFECTOR OF MLL FUSION PROTEINS TO SAFEGUARD GENOMIC INTEGRITY

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Background: Acute Myeloid Leukemia (AML) frequently harbors chromosomal rearrangements involving the Mixed Lineage Leukemia (MLL) gene. More than 65 different MLL fusion genes exist and many of them have been described to act as strong cancer drivers. While critical effectors of different MLL fusion proteins that are presumed to employ different mechanisms of oncogenic transformation.

Aims: To this end we prepare for a clinical test using autologous cultured RBC to test their in vivo stability. In parallel we develop methods for unlimited production of cultured RBC. An immortal source to produce in vitro cultured RBCs (iRBC), such as iPSCs would allow selection of ‘universal donor’ RBC, or provide an autologous end product with the absence of immune reactions.

Methods: Protein complexes of 7 molecularly distinct, affinity-tagged MLL-FPs (MLL- AF4, MLL-AF9, MLL-ENL, MLL-CBP, MLL-EEN, MLL-GAS7 and MLL-AF1p) were purified from stable cell lines allowing for inducible, single-copy true gene expression and characterized by mass spectrometry. Data analysis identified a comprehensive protein-protein interaction network, which was functionally interrogated by a subtractive shRNA screening approach. Validation experiments included detailed RNAi- and CRISPR/Cas9-mediated loss of function experiments in cell lines and primary cells in vitro and in vivo, using readouts for changes in proliferation, differentiation, apoptosis and DNA damage.

Results: Characterization of the protein complexes nucleated by 7 MLL fusion proteins by affinity purification coupled to mass spectrometry (AP-MS) revealed a densely interconnected protein-protein interaction network of 963 proteins, comprising previously known MLL-interacting protein complexes (such as PRC2 or SWI/SNF), as well as a high number of new interaction partners of MLL. 128 proteins were found to interact with ≥5 of all 7 MLL-fusions. This subset of conserved MLL-interaction partners was highly enriched for proteins with function in chromatin metabolism and transcriptional control. Systematic functional investigation of the conserved MLL-fusion interactome using subtractive shRNA screens identified the methyltransferase SETD2 as a critical effector of MLL fusion proteins. Both RNAi-based suppression and CRISPR/Cas9-mediated mutagenesis of SETD2 induced myeloid differentiation and apoptosis in human and mouse MLL-rearranged cell lines, while having only modest effects on the proliferation of MLL-wild type leukemia cells. Depletion of SETD2 in MLL-fusion- positive changes in proliferation, differentiation, apoptosis and DNA damage.

Summary/Conclusions: In summary, our data highlight the functional relevance of combined proteomic-genomic cellular screening to identify critical effectors of MLL-FPs. In addition, our study identifies a novel role for SETD2 in the maintenance of genomic integrity during initiation and progression of MLL-rearranged AML and establishes SETD2 as a therapeutic target in leukemia with low genenic complexity.

S134

CEBPA-MUTANT ACUTE MYELOID LEUKEMIA IS SENSITIVE TO SMALL-MOLECLE-MEDIATED INHIBITION OF THE MENIN-MLL INTERACTION

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Background: The CEBPA gene - encoding for the transcription factor C/EBPα - is mutated in 9% of patients with acute myeloid leukemia (AML). CEBPA N-terminal mutations lead to selective loss of full length C/EBPα p42 expression without affecting translation of a balanced of the shorter p30 isoform. As a balanced of C/EBPα isoforms is crucial for hematopoietic homeostasis, depletion of p42 leads to increased cell growth and blocks myeloid differentiation, resulting in the development of AML. We have recently shown that the p30 variant of...
C/EBPα can act as a gain-of-function allele with distinct molecular properties. However, the molecular basis of CEBPα p30-induced leukemogenesis is incompletely understood.

**Aims:** We hypothesized that the interaction between the oncogenic CEBPα p30 isoform and the MLL/SET histone methyltransferase complex is required for p30-dependent epigenetic and transcriptomic changes that contribute to leukemogenesis. Therefore, we aimed to investigate the sensitivity of CEBPα mutant MLL to perturbation of the MLL/SET function.

**Methods:** We used CRISPR/Cas9-mediated mutagenesis to interfere with the MLL/SET complex in myeloid progenitor cells from a Cebpap30/p30 AML mouse model. Cellular competition assays were used to assess changes in proliferative capacity of mutant AML cells. Further, MLL activity was inhibited by small molecules that block the Menin-MLL interaction. In both cases, proliferation, myeloid differentiation and apoptosis were used as readouts. Global changes in gene expression were measured by RNA-seq.

**Results:** We initially confirmed, via ChIP, that CEBPα and MLL co-localize on the CEBPα p30 isoform and the MLL (xenotransgenic) complex in geldanamycin-treated cells. Upon pharmacological perturbation of the MLL/SET complex, we used MI-463, a potent small-molecule inhibitor of the Menin-MLL interaction. Inhibitor treatment led to a time- and dose-dependent impairment of proliferation, induction of cell cycle arrest and increased apoptosis in Cebpap30/p30 cells. RNA-seq analysis showed that treatment induced expression patterns associated with metabolic reprogramming. Importantly, expression of CEBPα p30 was associated with hypersensitivity to Menin-MLL inhibition, as Cebpap30/p30 cells were 2-6 fold more sensitive than other leukemia cell lines of mouse and human origin.

**Summary/Conclusions:** We show that CEBPα mutant AML is highly sensitive to perturbation of the MLL/SET complex, either via genetic ablation of MLL or through pharmacological inhibition of the Menin-MLL interaction. Our data indicate that leukemic mutations of CEBPα selectively cooperate with the SET/MLL complex to regulate gene expression. These findings expand our understanding of and may inform new therapeutic strategies for N-terminal CEBPA mutated AML.

**S135**

**INHIBITION OF THE MYELOID MASTER REGULATOR PU.1 AS A THERAPEUTIC STRATEGY IN ACUTE MYELOID LEUKAEMIA**


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**Background:** FLT3 tyrosine kinase (TK) activating mutations (FLT3-ITD) are amongst the most frequent in AML and are associated with a poor outcome. FLT3-ITD promote constitutive activation of survival/proliferation pathways and the FLT3-TKD participates in the regulation of glycolysis and glutamine metabolism. The FLT3 TK represents a valid therapeutic target and several FLT3 TK inhibitors (TKI) have been developed. However despite showing activity in the preclinical setting, FLT3 TKI have displayed limited efficacy in clinical trials. Resistance mechanisms to FLT3 TKI include receptor mutations and cell intrinsic adaptive mechanisms. Amongst the latter, metabolic adaptations to targeted therapy in FLT3 mutated AML may play a significant role although the exact mechanisms are still ill-defined.

**Aims:** We hypothesized that metabolic adaptations facilitate FLT3 TKI resistance and aimed to identify early metabolic changes in FLT3-ITD AML, following TKI treatment, in an attempt to unveil novel therapeutic vulnerabilities.

**Methods:** Liquid chromatography coupled to mass spectrometry (LC/MS), using stable isotope-based carbon flux tracing, and oxygen consumption rate/extracellular acidification rate as measured by an extracellular flux analyser ( Seahorse, Agilent Technologies) were used to assess metabolic changes in FLT3-ITD AML cell lines. Metabolic adaptations were measured in the same conditions by RNA sequencing. Changes in viability and reactive oxygen species (ROS) in various culture conditions were measured by FACS. Gene silencing was performed using CRISPR-Cas9 gene editing and inducible short hairpin RNA interference.

**Results:** Analysis of published gene expression datasets demonstrated that glycolytic, citric acid cycle (CAC), and oxidative phosphorylation genes are upregulated in FLT3-ITD compared to FLT3 wild-type (FLT3 WT) patient samples at diagnosis. When we confirmed that both human and murine FLT3-ITD cells display increased glycolytic and respiratory capacity compared to FLT3 WT cells. Gene expression changes were validated in FLT3-ITD Gata1−/− mutant AML cells, which showed increased glycolysis. The FLT3 TK inhibition, glucose uptake was reduced. Metabolic flux analysis using [U-13C]glutamine demonstrated that glutamine, while providing carbons for the glycolysis, was primarily used for glutathione biosynthesis. In mouse mutant AML cells, glutaminolysis was decreased upon AC220 treatment. This antioxidant function is necessary because, as expected, FLT3-ITD cells displayed a large increase in ROS levels following TKI treatment when grown in the absence of glutamine and these changes correlated with a significant reduction in viability in the same condition. When treated with the high selective FLT3 TKI AC220, 8-fold increase in ROS levels which could be rescued by supplementation of the media with the antioxidant N-acetylcyesteine or a cell-permeable form of the CAC intermediate alpha-ketoglutarate.
Summary/Conclusions: Our data suggest that upon AC220 treatment, glutamine metabolism becomes a critical metabolic dependency in FLT3mutAML. Glutamine metabolism is mostly channelled towards glutathione production, while also supporting the CAC and both these fates contribute to its protective effects following FLT3 TK inhibition by respectively counteracting oxidative damage and sustaining macromolecule biosynthesis and cellular energetics. These data predict that a combined inhibition of glutamine metabolism and FLT3 TK activity may improve the eradication of FLT3mutAML cells.

Hematopoiesis, stem cells and microenvironment

S137

STEP-WISE REPROGRAMMING OF ENDOTHELIAL CELLS INTO IMMUNE-COMPETENT HEMATOPOIETIC STEM CELLS

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Background: The molecular pathways and microenvironmental cues that choreograph the conversion of endothelial cells (ECs) into long-term repopulating hematopoietic stem cells (HSCs) remain poorly defined. This is due to lack of models that recreate the ephemeral transition of an endothelial cell to a hemogenic state to the emergence of HSCs.

Aims: To reprogram adult mouse ECs into long-term repopulating HSCs that give rise to all hematopoietic lineages, including functional T cells in vivo. To provide a platform to deconvolute the process by which endothelial-to-hematopoietic transition is possible.

Methods: Here, we have developed a modular in vitro model in which—by precise, conditional expression of transcription factors: FosB, Gfi1, Runx1, and Sp1 (FGRS), and reintroduction of a proper inductive niche—adult mouse ECs were reprogrammed into HSCs (rEC-HSCs) with multi-lineage engraftment potential (rEC-MPPs). Adult, non-lymphatic ECs isolated from various organs of Runx1-IRES-GFP reporter mice were transduced with FGRS and co-cultured in direct contact with vascular niche.

Results: Within 14 days, ECs initiated a hematopoietic program, turning on the endogenous expression of Runx1 and transitioning into hematopoietic cells. Expansion of these cells for another 14 days resulted in generation of rEC-HSCs and rEC-MPPs. Transplantation of rEC-HSCs and rEC-MPPs (CD45.2+) into lethally irradiated mice (CD45.1+) reconstituted both short-term (rEC-MPPs) and long-term hematopoiesis, with secondary engraftment potential (rEC-HSCs). rEC-HSCs gave rise to both functional myeloid and lymphoid cells with full complement of polarized T cell subsets. rEC-HSC-derived T cells undergo T-cell receptor (TCR) rearrangement and restore adaptive immune function in Rag1−/− mice.

Summary/Conclusions: This multi-phasic, step-wise approach provided an interrogable model to decipher pathways involved in EC transition into hematopoietic cells. This will provide cues to devise strategies to convert autologous ECs into large numbers of HSCs for genetic modification and subsequent treatment of both genetic and acquired hematological disorders.

S138

MARROW MESENCHYAL STEM CELLS RESCUE BONE MARROW ENDOTHELIAL CELLS SUFFERING CHEMOTHERAPY STRESS BY TRANSFERRING MITOCHONDRIA THROUGH NANOTUBES

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Background: The tunneling nanotube (TNT) is a newly discovered, long and thin tubular structure between cells and can facilitate the intercellular exchange of diverse cellular signals and components ranging from electrical signalling to organelles. Recent reports show that mesenchymal stem cells (MSC) rescue injured target cell and promote target cell recovery from a variety of stress including oxidative stress, ultraviolet radiation, ischemia/reperfusion (I/R)etal. However, it is still unclear if bone marrow mesenchymal stem cells (BMMSC) can also form TNT to communicate and rescue injured bone marrow -derived endothelial cells (BMdEC)and promote it recovery from chemotherapy stress. In our study, we set out to test the hypothesis that BMMSC can rescue suffering endothelial cells by transferring mitochondria to endothelial cells through nanotubes.

Aims: To investigate the novel intercellular communication TNT between BMMSC and BMdECs or HUVEC, illuminating its constituent and investigating the significance of transport of mitochondrial through TNT between BMMSC and BMdECs or HUVEC suffering from chemotherapy stress of cytosine arabinoside.

Methods: We established two direct co-culture system for human primary bone marrow mesenchymal stem cells (BMMSCs) and bone marrow-derived endothelial cells (BMdECs) or Human umbilical cord vein endothelial cells (HUVECs) respectively.

Results: Firstly, We observed the TNTs formed between BMMSCs and endothelial cells including the TNT structure between BMMSCs and HUVECs or BMdECs are composed with F-actin, microtubule in addition to membrane. Live cell imaging showed the two xeno-genetic cells form TNTs by retaining a thin thread of membrane upon dislodge-
S139

SHORT-TERM FEEDING OF A HIGH-FAT DIET DISTURBS LIPID RAFT/TGF-BETA SIGNALING-MEDIATED QUIESCENCE OF HEMATOPOIETIC STEM CELLS IN C57BL/6J MOUSE BONE MARROW

Background: In vivo and in vitro studies showed that feeding a HFD affects hematopoiesis in bone marrow (BM), with a decreased proliferation of hematopoietic cells. However, it is currently difficult to say whether these alterations are related to direct effects such as changes in lipid metabolism in HSC or indirect "side effects" on HSC, such as pathophysiology related to obesity or inflammation. In this study, we observed after an extended diet over several months or a diet very rich in fat (>60 kJ% of fat). For example, HFD-induced obesity significantly alters hematopoiesis in bone marrow (BM), with a decreased proliferation of HSC, a general suppression of progenitors, an enhancement of lymphopoiesis, and an activation of myeloid cell production from BM progenitors. Inflammation also affects HSC homeostasis, as interferon alpha is well-known to activate proliferation inhibition and alter its formation of capillary-like structures.

Our study offers the clues to help know about cell-cell communication of niche components in the HSC niche in bone marrow.

Aims: Our strategy is to characterize the impact of a short-term HFD on HSC and hematopoiesis in non-obese C57BL/6J mice.

Methods: In a prospective study, C57BL/6J mice were fed a control diet (4 kJ% of fat) or a HFD (42 kJ% of fat), over a short period of 4 weeks, to investigate the direct impact of such a diet on hematopoiesis.

Results: While fat intake led to an increase in plasma cholesterol levels, mice did not develop obesity, and no inflammatory monocytes and no modulation of pro- and anti-inflammatory cytokine levels were detected in blood and BM, respectively. A major impact was observed on the lymphopoiesis, with a very rich in fat (>60kJ% of fat). For example, HFD-induced obesity significantly alters hematopoiesis in bone marrow (BM), with a decreased proliferation of HSC, a general suppression of progenitors, an enhancement of lymphopoiesis, and an activation of myeloid cell production from BM progenitors. Inflammation also affects HSC homeostasis, as interferon alpha is well-known to activate dormancy of HSC in vivo.

Summary/Conclusions: From these results, we hypothesized that HFD could induce major perturbations in murine hematopoietic stem cells (HSC) and hematopoietic system homeostasis. It is, however, difficult to say whether these alterations are related to direct effects such as changes in lipid metabolism in HSC or indirect "side effects" on HSC, such as pathophysiology related to obesity or inflammation.

S140

A NOVEL MODEL OF HUMAN LYMPHO-MYELOID PROGENITOR HIERARCHY BASED ON SINGLE CELL FUNCTIONAL AND TRANSCRIPTIONAL ANALYSIS


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Background: Human hematopoiesis produces 10 billion new, terminally mature, blood cells daily, a production that is also rapidly responsive to external change. Dysregulation of this complex process can lead to hematopoietic and immune deficiencies and blood cancers. In humans, the hematopoietic progenitor hierarchy producing lymphoid and granulocytic-monocytic (myeloid) lineages is unclear. Multiple progenitor populations give rise to lymphoid and myeloid cells but remain incompletely characterized at the immunophenotypic, transcriptional and functional level.

Aims: Here, we aimed to understand the clonal functional output and transcriptional programs of current primary human lympho-myeloid containing progenitor populations - the lymphopoiesis, the lymphoid-driven primed multi-potential progenitor (LMP1), the multi-potential progenitor (LMP2) and granulocyte-macrophage progenitor (GMP).

Methods: We devised a FACS-staining and sorting strategy to prospectively purify eight human hematopoietic stem and progenitor cell (HSPC) populations. We compared functional output of LMP, GMP and GMP in vitro by quantitative CFU assays, single cell liquid cultures or limit dilution analysis and in vivo by transplantation into humanized oseils. We performed RNA sequencing and single cell RT-PCR analysis to understand the relationship between functional and transcriptional heterogeneity.

Results: Our study comprehensively characterized the LMP, GMP and LMP2 of human lymphoid and myeloid populations. Both LMP and GMP are very rare within the mononuclear fraction (1 in 10^4 to 1 in 10^5). We cultured 3806 single LMP, GMP and LMP2 cells (isolated from 21 cord blood units and equivalent to ~10^11 mononuclear cells) under three different culture conditions. We observed marked functional heterogeneity in the three lympho-myeloid progenitor populations - the single cell output of T lymphoid progenitor (LMP1), the multi-potential progenitor (LMP2) and granulocyte-macrophage progenitor (GMP). Notably, the rescue effect was inhibited when the formation of TNTs were impaired by incubating with an F-actin-depolymerizing drug and tubulin -depolymerizing drug, indicated that these TNTs transferring mitochondria have a distinct cytoskeletal composition which is important to deliver the functional mitochondria from the TNT to interact with untreated BMMSCs and then mesenchymal stem cells to restore the functionality of HSC. Not only our results uncover the impact of HFD independently of obesity but they also identify the disturbance of LR/TGF-beta signaling-mediated quiescence as its main molecular mechanism of action.

References

DK and BS are equally contributing authors.
Gene therapy, immunotherapy and vaccination 1

S141

WILMS’ TUMOR 1 RNA-ECTROPLATED DENDRITIC CELL VACCINATION AS POST-REMISSION TREATMENT TO PREVENT OR DELAY RELAPSE IN ACUTE MYELOID LEUKEMIA: FINAL RESULTS OF A PHASE II STUDY IN 30 PATIENTS

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Background: Relapse is a major problem in acute myeloid leukemia (AML) and adversely impacts survival.

Aims: The aim of this phase II study was to determine the clinical efficacy of dendritic cell (DC) vaccine therapy in AML, and, more specifically, whether this form of immunotherapy can be applied in the post-relapse adjuvant setting to decrease the risk of relapse following chemotherapy and to improve survival.

Methods: We vaccinated 30 AML patients in remission following polychemotherapy, but at very high risk of relapse with autologous DCs loaded with the AML-associated MT1 (WT1) antigen by means of mRNA electroporation, a technique that allows for human leukocyte antigen haplotype-independent, multi-epitope antigen presentation to T-cells. The vaccines were administered intradermally. WT1 mRNA levels in blood and marrow were followed as a measure of minimal residual disease. Circulating WT1-specific CD8+T-cells obtained between 14 and 21 days after the DC vaccination were stained with WT1-HLA-A*0201 tetramers.Tos assess cell-mediated immunity in vivo, delayed type hypersensitivity (DTH) skin testing was performed 2 weeks after the 4th DC vaccination by intradermal injection; DTH-infiltrating lymphocytes collected from skin biopsies were expanded for 2-3 weeks in medium with interleukin-2, human CD3/CD28 beads and WT1 specificity and reactivity.

Results: There was a demonstrable anti-leukemic response in 13/30 patients (overall response rate 43%). Nine patients achieved molecular remission as demonstrated by normalization of WT1 transcript levels, 5 of which are sustained after a median follow-up of 109.4 months, including 1 patient who went from relapse to complete remission by DC vaccination only. In the remaining 4 responding patients, the clinical response was characterized by stable disease as demonstrated by elevated but stable WT1 transcript levels in blood for 3-12 months and stable blood values without blasts. Five-year overall survival was 40%, as compared to 24.7% in the SEER data of the National Cancer Institute; it was significantly higher in responders than in non-responders (53.8% vs 25.0%; p=0.01). In patients receiving DCs in first complete remission (CR1), there was a vaccine-induced relapse reduction rate of 25% and the 5-year relapse-free survival was significantly higher in responders than in non-responders (50% vs 7.7%; p=0.0001). In patients ≤55 and >65 years who received DCs in CR1, 5-year overall survival was 69.2% and 30.8% respectively. Of the 30 patients, 11 are alive in CR, including ≤65 and >65 years who received DCs in CR1, 5-year overall survival was 40%, as compared to 24.7% in the SEER data of the National Cancer Institute.

Summary/Conclusions: Vaccination of AML patients with WT1 mRNA-electroporated DCs can be an effective and non-toxic strategy to prevent or delay leukemia relapse after standard chemotherapy, translating into improved overall survival rates, which are correlated with the induction of WT1-specific CD8+ T-cell responses.

S142

FIRST-IN-HUMAN MULTICENTER STUDY OF BB2121 ANTI-BCMA CAR T CELL THERAPY FOR RELAPSED/REFRACTORY MULTIPLE MYELOMA: UPDATED RESULTS

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Background: To test the safety and efficacy of the CAR T cell modality in relapsed/refractory multiple myeloma (MM), we have designed a second-generation CAR construct targeting B cell maturation antigen (BCMA) to redirect T cells to MM. bb2121 consists of autologous T cells transduced with a lentiviral vector encoding a novel CAR incorporating an anti-BCMA scFv, a 4-1BB costimulatory motif and a CD3-zeta T cell activation domain. We will report updated safety and efficacy results following promising initial results (Berdeja et al. ENA 2016).

Aims: The primary objective is to determine the maximally tolerated dose of bb2121 in subjects with MM whose tumors express BCMA, to determine and test a recommended phase 2 dose for future studies. The secondary objective is to provide preliminary efficacy data on the anti-tumor effects of treatment with bb2121 in subjects with MM whose tumors express BCMA.

Methods: CRB-401 (NCT02656992) is a multi-center phase 1 dose escalation trial of bb2121 in patients with relapsed and/or refractory MM who have received ≥3 prior regimens, including a proteasome inhibitor and an immunomodulatory agent or other double-refractory. BCMA expression by bone marrow aspirate cells. Peripheral blood mononuclear cells are collected via leukapheresis. Patients undergo lymphodepletion with Flu (30 mg/m2) Cy (300 mg/m2) daily for 3 days then receive 1 infusion of bb2121. The study follows a standard 3+3 design with planned dose levels of 5.0, 15.0, 45.0, 80.0 and 120 x 107CAR+T cells.

Results: As of November 18, 2016, 11 patients had been infused with bb2121 in the first 4 dose cohorts, and 9 patients had reached at least 1 month of follow-up. As of data cut-off, no dose limiting toxicities, and no >Grade 2 neurotoxicities or cytokine release syndrome (CRS) had been observed. Grade 1-2 CRS has been reported in 8/11 (73%) treated patients. All patients treated with doses ≥25.0% or higher had ≥Gr3 CRS occurring up to 10 days after the last dose by day 21. Disease Burden Reduction (DBR) was defined as the percentage decrease of minimal residual disease. Circulating WT1-specific CD8+T-cells obtained from skin biopsies were expanded for 2-3 weeks in medium with interleukin-2, human CD3/CD28 beads and WT1 specificity and reactivity.

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S143

BASELINE AND EARLY POST-TREATMENT CLINICAL AND LABORATORY PARAMETERS ASSOCIATED WITH SEVERE NEUROTOXICITY FOLLOWING 19-28Z CAR T CELLS IN ADULT PATIENTS WITH RELAPSED B-ALL

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Background: CD19-specific chimeric antigen receptor (CAR) modified T cells produce high anti-tumor activity in relapsed or refractory (R/R) ALL, but can be associated with cytokine release syndrome (CRS) and neurotoxicity (NTX). Aims: We examined baseline and post-treatment clinical and laboratory parameters to identify factors associated with severe NTX (≥Grade 3) in our phase I clinical trial of CD19-specific 19-28z CAR T cells for adult patients (pts) with R/R B-ALL (NCT01044069).

Methods: In 51 adult pts with R/R B-ALL were treated with 19-28z CAR T cells following conditioning chemotherapy at MSKCC. In order to identify clinical and serum biomarkers associated with severe NTX (≥NTX), we examined demographic, treatment, and clinical blood parameters as well as in vivo CAR expansion and serum cytokines, and performed univariate and multivariate analysis.

Results: In this cohort of ALL pts, 20, 8, 2, 18, and 3 pts experienced Gr 0, 1, 2, 3, and 4 NTX, respectively. No pts developed Grade 5 NTX and no cerebral edema was seen. Disease burden (≥50% blasts) at the time of T cell infusion (p=0.0045) and post-treatment ≥Gr3 CRS (p=0.0010) were significantly associated with ≥NTX, but we found no association with age, weight, T cell dose, choice of conditioning chemotherapy (Flu/Cy vs Cy), and prior lines of treatment. Among the clinical and blood parameters, fever, low PLT, high ferritin and MCHC as well as elevated GM-CSF, IFNγ, IL-15, IL-5, IL-10, IL-2 at day 3 of T cell infusion at day 3 of T cell infusion were significantly associated with ≥NTX (all p<0.01). While some of these cytokines were also elevated in severe CRS cases, IL-2 and IL-10 at day 3 are unique to ≥NTX. Furthermore, in vivo peak CAR T expansion at day 7 (p=0.001) significantly correlated with ≥NTX (p<0.01). Lastly, multivariate analysis revealed baseline PLT <60 or MCHC ≥33.2 and morphologic disease (>5% blasts) has 95% sensitivity and 70% specificity of identifying ≥NTX pts.

Summary/Conclusions: These data provide a characterization of early clinical and serum biomarkers of ≥NTX in adult pts receiving 19-28z CAR T cells and should help identify appropriate pts for early intervention strategy to mitigate NTX.

S144

FIRST-EVIDENCE DEMONSTRATING ENRICHMENT AND REPOPULATING ADVANTAGE OF GEN-CORRECTED HEMOPoietIC REPOPULATING CELLS IN NON-CONDITIONED FANCONI ANEMIA PATIENTS


haematologica | 2017; 102(s2) | 21
Background: Fanconi anemia (FA), is a monogenic inherited syndrome asociated with bone marrow failure (BMF), that has been considered a candidate disorder for hematopoietic stem cell (HSC) gene therapy. To date, three clinical trials have been performed, all of which failed to demonstrate engraftment of corrected HSCs.

Aims: To demonstrate engraftment of gene-corrected HSCs in non-conditioned Fanconi anemia patients.

Methods: To improve previous results, we proposed a new approach based on two clinical trials. First, to increase the HSC collection, we designed a trial employing a plerixafor plus G-CSF mobilization regimen. Second, to improve the quality of corrected HSCs, cells were pre-stimulated for only 8-10 hours and transduced with a new lentiviral vector (PGK-FANCA.Wpre) for 12-14h, a substantially shorter duration than in previous trials. To avoid chemotherapy-induced damage, a conditioning regimen was not included in the trial, based on the expected proliferative advantage of autologous corrected HSCs.

Results: Eight patients have been included so far in the HSC collection trial. No severe adverse events (SAE) related to the procedure have been reported. The most relevant AE has been the transfusion of packed red blood cells and platelets. Six FA patients aged 3-6 years underwent collections after mobilization of significant numbers of CD34+ cells (10 to 70 CD34+ cells/µL) to peripheral blood. Two patients (15 and 16 years) failed to mobilize. On average, 5 million CD34+ cells/Kg were collected, with 45% recovery after immunoselection. Engraftment of corrected HSCs was observed in the three patients.

Summary/Conclusions: Our preliminary results show that 1) HSCs collection is both safe and efficient in very young FA patients after mobilization with G-CSF and plerixafor, and 2) Engraftment and proliferation advantage of gene-corrected HSPCs has been demonstrated in FA patients even in the absence of conditioning regimens. The long-term follow-up of patients included in these clinical trials will demonstrate the feasibility of restoring the hematopoietic function of FA patients by means of a gene therapy approach in the absence of conditioning.

S145

TARGETING FLT3 WITH CHIMERIC ANTIGEN RECEPTOR T CELLS CONFER POTTENT REACTIVITY AGAINST ACUTE MYELOID LEUKEMIA

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Background: Adoptive immunotherapy with chimeric antigen receptor (CAR)-modified T cells has therapeutic potential in hematologic malignancies. We are pursuing FLT3-ITD as a novel CAR target in acute myeloid leukemia (AML). FLT3 is a homodimeric transmembrane protein with uniform expression on AML, irrespective of cytogenetic and histomorphologic subtype. FLT3 provides survival signals to AML blasts and is a key driver of leukemia-genesis in AML cases with internal tandem duplication (FLT3-ITD). These attributes suggest FLT3 may be an ‘Achilles heel’ making AML blasts susceptible to CAR T-cell mediated recognition and elimination.

Aims: We therefore explored the anti-leukemia efficacy of FLT3-CAR modified T cells against FLT3-ITD+ and FLT3 wild type AML in pre-clinical models in vitro and in vivo.

Methods: A FLT3-CAR comprising a single-chain variable fragment (4G8), fused to an IgG-Fc spacer, and signaling module with CD3 zeta and CD28 was encoded in a lentiviral vector (epHIV7) for gene-transfer into CD8+ and CD4+ T cells of healthy donors (n>4) and AML patients. CAR T-cell mediated cytolytic activity was evaluated in FACS-fluorescence-based assays, cytokine production analyzed by ELISA and proliferation assessed by CFSE dye dilution. Immunodeficient NSG mice were engrafted with AML cell line (Molm-13) or primary AML blasts and treated with 5x10⁶ CAR-modified or control T cells (CD8:CD4 ratio=1:1).

Results: We confirmed specific recognition and high-level cytolytic activity of CD8+FLT3-CAR T cells against a panel of AML cell lines including THP-1 (FLT3 wild type), and Molm-13 (FLT3-ITD heterozygous). Both CD8+ and CD4+ FLT3-CAR T cells produced IFN-γ and IL-2, and underwent proliferation after antigen stimulation. FLT3-CAR T cells that we prepared from AML patients exerted specific anti-leukemia reactivity against autologous primary AML blasts, with near-complete cytolysis within 24 hours of co-culture. Further, FLT3-CAR T cells conferred a potent anti-leukemia effect in vivo models of systemic leukemia, both with AML cell lines (Molm-13) and primary AML blasts. A single dose of FLT3-CAR T cells conferred complete eradication of leukemia from peripheral blood, bone marrow and spleen, as confirmed by bioluminescence imaging and flow cytometry. FLT3 is not expressed in any normal solid tissues and mature hematopoietic cells, but shows limited expression in hematopoietic progenitors and hematopoietic stem cells (HSCs). Preliminary data show that FLT3-CAR T cells recognize FLT3+/high normal HSCs and interfere with normal hematopoiesis, but preserve a proportion of HSCs capable of reconstituting hematopoietic lineages. Studies to assess recognition of normal HSCs in vivo are ongoing.

Summary/Conclusions: Collectively, our data demonstrate that T cells expressing a FLT3-specific CAR mediate potent reactivity against FLT3 wild type and FLT3-ITD+A ML in vitro and in vivo, and establish FLT3 as a novel CAR target in AML. FLT3-ITD positivity identifies a high-risk AML subgroup that may particularly benefit from adoptive therapy with FLT3-CAR T cells, e.g. in order to achieve ‘minimal residual disease’ (MRD) negativity prior to allo-geneic HSC transplantation. Our data further suggest that in contrast to CD33 and CD123, which are pursued as alternative CAR targets in AML, targeting of FLT3 may preserve a fraction of normal HSC and enable the implementation of CAR therapy outside the transplant setting.
**PRESIDENTIAL SYMPOSPUM**

**BEST ABSTRACTS**

**S146**

**BPX-501 DONOR T CELL INFUSION WITH INDUCTIBLE CASPASE 9 SUICIDE GENE FACILITATES HLA-HAPLOIDENTICAL STEM CELL TRANSPLANTATION IN CHILDREN WITH BOTH HEMATOLOGICAL MALIGNANT AND NON-MALIGNANT CONDITIONS**

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**Background:** Allogeneic hematopoietic hematopoietic stem cell transplantation (HSCT) offers curative therapy for children who lack an available HLA-identical donor with hematopoietic disorders such as primary immune disorders (PID), hemoglobinopathies, erythroid disorders and acute leukemias. qβ T-cell depletion mitigates the risk of GVHD after haplo-HSCT, but is associated with extended immunodeficiency, leading to complications due to infections. We have performed qβ TCR-depleted haplo-HSCT with post-transplant infusion of BPX-501 gene modified T cells to allow for more rapid immune reconstitution. Upon occurrence of GVHD, administration of rimiducid (ApR1903) dimerizes the Caspase 9 suicide switch and rapidly induces apoptosis of the transduced BPX-501 cells and mitigates the GVHD.

**Aims:** This study was performed to determine the impact of BPX-501 T cell infusion on outcomes (treatment related mortality (TRM), disease recurrence, GVHD incidence and immune reconstitution) after HSCT.

**Methods:** We report on a large multicenter, prospective Phase I-II study enrolling children receiving qβ T-cell depleted Haplo-HSCT. Patients were infused with BPX-501 T cells 2 weeks post-transplant. 104 patients have >100 day follow-up, 81 patients have follow up >180 days and 51 with >1 year follow-up. All patients received myeloablative therapy and low dose ATG prior to transplant. No pharmacologic Gvhd prophylaxis was given (Table 1).

**Table 1. Diagnoses of Patients with >100 day follow-up.**

<table>
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<tr>
<th>Type</th>
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<th>N=38</th>
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<tbody>
<tr>
<td>SCID</td>
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<td>ALL (CR1 CR2 CR3)</td>
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<tr>
<td>WAS</td>
<td>6</td>
<td>AMG</td>
</tr>
<tr>
<td>CDG</td>
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</tr>
<tr>
<td>MDY</td>
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<td></td>
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<tr>
<td>Sickle Cell</td>
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<td></td>
</tr>
<tr>
<td>Fanconi Anemia</td>
<td>8</td>
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<tr>
<td>HLI</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

**Results:** Cumulative incidence of TRM remains very low at 100 days (0%), 180 days (1.6%) and 1 year (2.8%). Of the 81 patients with >180 day follow-up, 20 patients had acute Gvhd 1-3 (24.7%) (Figure 1A); 10 with Grade 1, 8 with Grade 2 and 2 with Grade 3. Mild CDG was seen in 2 patients, moderate cGvhd in 2 patients and one case of severe cGvhd in a malignant patient, attributed to the allotagc, not BPX-501. Rimiducid was used in 4 patients with Grade 2 Gvhd with rapid resolution of symptoms, as it did in the severe cGvhd patient. In both malignant and non-malignant patients. CD3, CD4, CD8 (Figure 2B) and B cells (Figure 3C) immune reconstitution was brisk. CD3+CD19+ T-cells were detectable at one year via flow cytometry analysis in peripheral blood. In Wiskott-Aldrich patients, platelet recovery remains in the normal range at 180 days with mean platelet counts of 246.3±10^9/L. At 180 days and 1 year, the patients with hemoglobinopathies remain transfusion-free with a normal mean Hgb value of 11.4 g/dL.

**Summary/Conclusions:** These data suggest that infusion of BPX-501 modified T cells may facilitate T cell depleted Haplo-HSCT in children who would benefit from HSCT for either malignant or non-malignant conditions. The availability of a suicide gene mechanism in donor T cells infused after T depleted Haplo-HSCT, results in low rates of infection and rapidly reversible Gvhd when the dimension is infused to activate the suicide switch. Rapid cellular and humoral immune reconstitution makes BPX-501 after T depletion a safe and viable option for children who do not have a matched donor transplant and in whom transplantation has been deemed curative.

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**S147**

**RE-CREATING HEREDITARY PERSISTENCE OF FETAL HEMOGLOBIN WITH CRISPR/CAS9 TO TREAT SICKLE CELL DISEASE AND BETA-TALASSEMIKA**

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**Background:** Extensive human genetic and epidemiological data demonstrate that the genetic condition Hereditary Persistence of Fetal Hemoglobin (HPFH) substantially ameliorates the pathology of Sickle Cell Disease (SCD) and β-thalassemia (β-Thal). This condition is associated with several genetic variants at the β-globin locus that lead to transcriptional reactivation of γ-globin genes, resulting in upregulation of fetal hemoglobin (HbF).

**Aims:** CRISPR/Cas9 is a revolutionary technology that allows for precise, directed changes to genomic DNA. Our strategy is to use CRISPR/Cas9 in human primary CD34+ hematopoietic stem and progenitor cells (HSPCs) to re-create specific HPFH genetic variants as well as other variants associated with elevated HbF and demonstrate their causal relationship to elevated HbF as a potential therapeutic strategy to treat SCD and β-thal.

**Methods:** Using CRISPR/Cas9 gene editing, we have successfully re-created genetic variants linked to high HbF levels in HSPCs from healthy donors and SCD and β-Thal patient samples, and determined the relationship of different genetic variants to upregulation of γ-globin in bulk and clonal populations of differentiated erythrocytes. Off-target editing was assessed, and on-target editing in long-term repopulating subsets of HSPCs was measured in vitro and by engraftment in immunocompromised mice. Finally, editing rates at clinical scale in a GMP-capable manufacturing facility were demonstrated.

**Results:** We first optimized cell culture and electroporation conditions that lead to high rates of genomic editing across multiple loci, achieving >84%±6.2% (Mean±SD) editing efficiency at key regions of interest in CD34+ HSPCs from mobilized peripheral blood of healthy donors (n=16). Similar rates of editing were attained using CD34+ HSPCs derived from healthy-donor bone marrow (n=6). Cas9 delivery as recombinant protein improved cell viability when compared to mRNA-based delivery (98.5±3.7% compared to 75.5±3.3%, Mean±SD, n=56 for each) with no observed reduction in editing efficiencies. To investigate gene editing impact on Hbf, edited cells were erythroid differentiated from healthy donors as well as from SCD and β-Thal patients. Specific gene edits significantly increased γ-globin mRNA expression to therapeutically-relevant levels (increased expression to 29-37% as a ratio of γ/ε in one β-Thal patient sample and to 25-45% as a ratio of γ(ε)+β in six SCD patient samples). We demonstrated similarly high rates of editing in the CD34+CD38 CD90 CD45RA+ long-term repopulating HSPCs and bulk CD34+ HSPCs (87.9±6.4% compared to 89.7±5.6%, Mean±SD, n=48 for each). We confirmed that editing levels of edited cells in immunocompromised mice were similar to control cells (% human CD45 in peripheral blood = 28.6±9.9% in controls versus 27.1±6.6% and 26.3±7.9% for two guide targets, Mean±SD, n=48 for each).

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haematologica | 2017; 102(s2) | 23
for a selected guide RNA confirmed no detectable genomic cleavage at over 500X fold target site with a detection sensitivity of 0.2%, supporting its safety for clinical use. Finally, we have demonstrated editing rates of >85% at clinical scale in a GMP-capable manufacturing facility to enable clinical development for SCD and β-Thal. Required safety toxicology studies are ongoing. 

Summary/Conclusions: Using CRISPR/Cas9 we successfully created gene editing models to upregulate HbF in both healthy donor and patient samples. We have also dissected the genotype-phenotype relationship for specific genetic modifications, identifying the editing strategies which are most promising for expressing HbF. We have optimized the conditions for modifying HSPCs, including at clinical scale in a GMP-compliant setting, and demonstrated potential minimal off-target editing. These experiments support the further development of specific CRISPR/Cas9 editing strategies of HSPCs to treat SCD and β-Thal patients.

S148

EXPOSURE TO INFECTION TRIGGERS PAX5 AND ETV6-RUNX1 CHILDHOOD B-ALL

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Background: B-cell precursor acute lymphoblastic leukemia (BCP-ALL) of childhood remains a major cause of death in high-income countries. It has a yet unexplained peak incidence between 2-6 years of age and a potential trigger was theorized a century ago with several possibilities of exposure to infection in infancy. Recently in vitro and in vivo evidence strengthened the causal role of exposure to infection in BCP-ALL (1, 2). However, it remains unknown which specific exposure precede BCP-ALL development. We hereby explore the role of infection and how the pre-leukemic clone evolves to BCP-ALL.

Aims: Aiming to understand the role of infection exposure in the etiology of childhood BCP-ALL.

Methods: We have developed and characterized two independent GEMMs, in addition to the Pax5+/- infection model (1), which were exposed to a common infection environment. These represent childhood BCR-ABL+190 BCP-ALL and the most common subtype ETV6-RUNX1 BCP-ALL. Both model systems ensure Sca1-directed expression of BCR-ABL+190 or ETV6-RUNX1 in HSC/PC and not in B-lineage cells. We additionally outlined the potential role of the next HSC/PC subsets in the development of BCP-ALL independent of exposure to common infection. The molecular mechanism leading to BCP-ALL identified in the infection dependent GEMMs is determined by the genetic predisposition (Pax5+/- or ETV6-RUNX1). Pax5+/- mice acquire constitutive activating Jak3 mutations (6/9) in a susceptible B-cell precursor population (approximate immature B cell) (1). On the other hand Sca1+ ETV6-RUNX1 mice develop BCP-ALL at a low penetrance as well as wild-type mice (10.75%; 10 out of 93) with a CD19+B220+IgM-cell surface phenotype and manifested with blast cells in the peripheral blood (PB) and clonal immature BCR rearrangement.

Results: In vivo and in vitro infection of Pax5+/- and Sca1+ETV6-RUNX1 mouse models develop BCP-ALL only after exposure to common infections whereas the BCR-ABL+190 mice develop BCP-ALL independent of exposure to common infection. The molecular mechanism leading to BCP-ALL identified in the infection dependent GEMMs is determined by the genetic predisposition (Pax5+/- or ETV6-RUNX1). Pax5+/- mice acquire constitutive activating Jak3 mutations (6/9) in a susceptible B-cell precursor population (approximate immature B cell) (1). On the other hand Sca1+ ETV6-RUNX1 mice develop BCP-ALL at a low penetrance as well as wild-type mice (10.75%; 10 out of 93) with a CD19+B220+IgM-cell surface phenotype and manifested with blast cells in the peripheral blood (PB) and clonal immature BCR rearrangement. High expression of Recombination Activating Gene 1 (Rag1) and loss of function mutations in ETV6 are known for their association and role in human and murine B lymphomatisis (ETV6-RUNX1). Our study also supports the involvement of Rag1 in the human B cell development and our results may add a new layer of understanding to the genetic predisposition for BCP-ALL.

Conclusion: We demonstrated that activation of RHOA is pivotal for the development of BCP-ALL and a potential therapeutic target. The use of platelets in transfusion has increased dramatically in the last three decades. Cold temperature induces changes in glycosylation and clustering of platelet glycoprotein (GP) Ib and cytoskeletal rearrangements, which are recognized by host receptors resulting in lectin-mediated platelet aggregation and clearance in susceptible patients. Our study also suggests that current practice of platelet storage for transfusion uses room temperature and associates with a relatively high risk of bacterial growth and infection in susceptible patients.

Aims: Due to the cytoskeletal nature of the platelet changes upon refrigeration, we hypothesized that the RHO family GTPase activity is pivotal in the cold platelet lesion. Targeted intervention may benefit refrigerated platelets.

Methods: Analysis of RhoA, Rac1 and Cdc42 activity was performed using GST-Rhotekin and GST-PAK effector domain pull-down assays. Platelets were obtained from anticoagulated (CPD or EDTA) human, Rhesus monkey and murine whole blood. G0, NSC23766 and Casin, specific inhibitors for RhoA, Rac and Cdc42, respectively, were added and used at concentrations of 75 μM, 50 μM and 10 μM, respectively. RhoA deficient murine platelets were obtained from polycl-1 depleted Mx1-Cre;RhoAfl/+ mice. Aspirin was administered at a dose of 5 mg/Kg b.w to mice and monkeys. Bleeding time was performed using standard animal protocols. Transfused human/mouse platelets were compared to human or murine plasma or PAS-IV (67%/plasma/33%) at RT or 1-9°C for 7 days or 1-4 hours for murine platelets.

Results: We found that either short- or long-term refrigeration activates RHOA and RAC1, but not CDC42. Genetic deletion of RhoA or RhoA inhibition with the small molecule inhibitor G04 suffices to completely prevent cold-induced platelet clearance. After long-term cold storage of murine or human platelets, the effect of G04 is on-target since it mimics but does not modify the response of RhoA-deficient platelets. The effect of G04 is reversible since removal of G04 after 7-day storage restores RHOA activity to normal levels and allows normal extent of shape change and spreading on fibrinogen. To analyze the kinetics and hemostatic function of cold stored platelets, we analyzed the survival of xenotransfused human platelets after long-term (7-day) refrigeration in the presence and absence of inhibitors cocktail or individual inhibitors in macrophage depleted, sub-lethally irradiated NSG mice (N=20/group) as well as autologously transfused platelets in a crossover trial in Rhesus monkeys (N=5). Our results show that reversible inhibition of RHOA in refrigerated platelets suffices to survival levels similar to the unrefrigerated control in 100% of mice and 80% of monkeys (p<0.001). Our data further show that washing of platelets stored for 7 days in G04/plasma maintains collagen-induced shape change as well as normal aggregation of human platelets and restores bleeding time correction after congenic or autologous transfusion in all aspirated mice and 80% of aspirated Rhesus monkeys, respectively. RHOA inhibition blocks the process of intracellular traffic of GP through lipid rafts and endocytotic intermediates as assessed by confocal microscopy of GpIb and the vascular sorting protein VPS33b, as well as biochemical fractionation of detergent-insoluble membrane lipid raft and plasma membrane GPIb after reduced blebbing and formation of microparticles upon storage in G04/plasma.

Summary/Conclusions: We demonstrate that activation of RHOA is a pivotal mechanism of refrigerated platelet storage lesion and phagocytosis. Reversible inhibition of RHOA allows the extended cold storage of platelets which are effective in vitro and in vivo, suitable for use in clinical safety and efficacy trials. Our study also provides the mechanism and a stringent proof-of-principle for the translational application of a novel approach to refrigerated platelet storage.

S150

TREATMENT REDUCTION IN PATIENTS WITH ADVANCED-STAGE HODGKIN LYMPHOMA AND NEGATIVE INTERIM PET: FINAL RESULTS STORED IN INTERNATIONAL RANDOMIZED PHASE 3 TRIAL HD81 BY THE GERMAN HODGKIN STUDY GROUP

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Background: The German Hodgkin Study Group (GHSG) applies the intensive eBEACOPP regimen (dose-escalated bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone) to all newly diagnosed advanced-stage HL patients regardless of their individual risk-profile. However, some patients might not be in need of such an intensive treatment to achieve cure. Unfortunately, baseline risk factors as defined in the international prognostic score cannot identify these patients reliably. Recent clinical research suggests that early metabolic response assessment after 2 cycles of therapy using FDG-PET (PET-2) can better predict the individual outcome. In particular, a rapid response as determined by PET-2 negativity might allow reducing the overall treatment intensity.

Aims: To assess the feasibility of decreasing the number of eBEACOPP cycles in patients with negative PET-2 without loss of efficacy as determined by progression-free survival (PFS).

Methods: Between 05/2008 and 07/2014, we recruited patients with newly diagnosed, advanced-stage HL aged 18–60 years. All patients gave written consent before study entry. PET-2 was centrally assessed with FDG uptake not higher than the mediastinal blood pool defined as negative. Patients with negative PET-2 were randomly assigned to receive 6 or 2 additional cycles (i.e., 8 or 4 cycles of eBEACOPP in total, respectively). PET-positive residues after chemotherapy were irradiated. Based on the results of our previous HD15 trial, the protocol was amended in June 2011 and the standard therapy was reduced from 8 to 6 cycles of eBEACOPP in total. The trial was designed to exclude inferiority of 6% or more of the experimental treatment (4 cycles of eBEACOPP) compared with the pooled standard treatment (8 or 6x cycles of eBEACOPP) at 5 years.

Results: We enrolled 2,101 patients. 1,005 patients with negative PET-2 were randomly assigned to either 8/6 cycles of eBEACOPP (n=504) or 4 cycles of eBEACOPP (n=501). With a median follow-up of 55 months, estimated 5-year PFS in the per-protocol set was 90.8% (87.9–93.7) with 8/6 cycles of eBEACOPP and 92.2% (89.4–95.0) with 4 cycles eBEACOPP (difference +1.4%, 95% CI -2.7–5.4, excluding the non-inferiority margin of -6%). In the standard arm, 95% of patients had at least one acute hematological toxicity of CTCAE grade 3-4 compared with 90% in the experimental arm, including severe infections in 75 (15%) and 38 (8%), respectively. Acute severe organ toxicities were documented for 91 (18%) and 38 (8%), respectively. 25 patients (5%) in the standard group (8/6 cycles of eBEACOPP and 9 (2%) in the experimental group (4 cycles of eBEACOPP) died; most frequent cause of death was second malignancy (11 and 1 patient, respectively). No patient in the experimental group died from treatment-related toxicities. Estimated 5-year overall survival (OS) in the per-protocol set was 95.4% (93.4–97.4) with standard eBEACOPP, and 97.7% (96.2–99.3) with 4 cycles of eBEACOPP (log-rank p=0.004).

Summary/Conclusions: Metabolic response assessment using FDG-PET after 2 cycles of eBEACOPP allows the reduction from therapy with 8/6 to only 4 cycles without loss of efficacy as determined by PFS in advanced-stage HL patients. Furthermore, the abbreviated treatment with 4 cycles of eBEACOPP is associated with improved tolerability and consequently leads to a significant OS benefit over standard therapy. PET-guided reduced therapy with eBEACOPP combines outstanding efficacy with high safety. We therefore recommend this treatment strategy for advanced-stage HL patients.
Acute lymphoblastic leukemia - Biology 1

P151
TARGETED SINGLE CELL SEQUENCING TO IDENTIFY MUTATIONAL HIERARCHY IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA
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Background: Acute lymphoblastic leukemia (ALL) is a common childhood malignancy caused by clonal proliferation of immature B or T lymphoid cells. ALL patients are primarily young children who respond well to chemotherapy, with survival rates above 85%. However, if relapse develops, survival rates drop to 15-50%. Recent studies have shown that at diagnosis, different ALL subtypes can be identified that are likely the result of clonal branched evolution. Understanding this clonal evolution and the order in which mutations are acquired can provide improved insights into the origins of leukemia relapse.

Aims: To use single-cell sequencing to investigate (i) the heterogeneity of leukemic T-ALL cells present at diagnosis and (ii) unravel the order in which mutations were acquired during leukemia evolution.

Methods: Bone marrow samples taken at diagnosis and remission from 4 T-ALL patients underwent whole genome and RNA sequencing. Somatic mutations, indels and chromosomal translocations were confirmed using Sanger sequencing. Primers were designed to specifically target these genetic alterations, and included 46 primers against heterozygous SNPs for quality control assessment. A total of 1517 single cells (average of 379 cells per patient) were sorted using flow cytometry or a microfluidic device and analyzed with targeted sequencing. Cells were discarded from further analysis if focus and allelic drop-out exceeded 33.3%. Jaccard hierarchical clustering was applied to identify subclones and a new graph-based algorithm was developed to determine the order of mutation acquisition. Single CD34+CD38− HSPCs (hematopoietic stem/progenitor cells) were sorted to test for the presence of mutations in early progenitors.

Results: We detected between 2 and 4 separate clones in each T-ALL patient sample. Each patient harboured one dominant clone comprising 46 to 98% of all single cells that was highly mutated, accompanied by a number of smaller subclones carrying fewer mutations. No mutually exclusive mutations, fusion genes or deletions were observed between the clones arguing against inde- pendent leukemic clonal initiation events. Instead, a more sequential clonal hierarchy became likely, with each clone harbouring more mutations than the last. Using our newly developed graph-based algorithm, we found that early mutations mostly occurred in genes of unknown significance and may represent a pre-leukemic state. Three out of four patients also had an earlier mutation event in a known oncogene (MED12, STAT5B or NOTCH1). Intermediate events were detected in subclones and a new graph-based algorithm was developed to determine the order of mutation acquisition. Single CD34+CD38− HSPCs (hematopoietic stem/progenitor cells) were sorted to test for the presence of mutations in early progenitors.

Summary/Conclusions: We demonstrate that T-ALL patients have limited heterogeneity at diagnosis and that targeted single cell sequencing can be used to determine the cell of origin and the order of mutation acquisition. These data also illustrate that HSPCs at remission carry a few early, pre-leukemic events, while highly mutated HSPCs are eradicated during treatment, which is in line with long term remission in T-ALL.

P152
BCL-2 INHIBITION AS NEW THERAPEUTIC OPPORTUNITY FOR RPL10 R98S MUTANT PEDIATRIC T-ALL
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Background: The ribosomal protein L10 (RPL10) R98S mutation occurs in 8% of pediatric T-cell acute lymphoblastic leukemia (T-ALL) cases. RPL10 R98S leads to a proliferation defect in lymphoid cells but its oncogenic contribu- tion in pediatric T-ALL remains unclear. Treatment intensification and risk stratification has reduced the relapse rate of T-ALL to ~15% but further improve- ments will require strategies that focus on specific subtypes as RPL10 R98S; if the long-term sequela of toxic therapy are to be avoided.

Aims: (1) Explore the oncogenic contribution of the RPL10 R98S mutation in pedi- atric T-ALL. (2) Define new therapeutic opportunities for RPL10 R98S defective T- ALL. (3) Identify a biomarker indicative of the RPL10 R98S mutation in T-ALL.

Methods: Quantitative label-free proteomics was used to screen for protein differ- ences between RPL10 WT and R98S expressing Ba/F3 cells. Hits were con- firmed by western blot. Enzyme-linked immunosorbent assay (ELISA) bone marrow (BM) cells extracted from RPL10 WT and R98S knock-in mice and in RPL10 WT and R98S pediatric T-ALL samples. Serial re-plating was established by plating 2000 cells/ml in Methocult. Oxidative stress and mitochondrial activity was determined by Dihy- droethidium and mitotracker. Viable cell counts were determined by Annexin V exclusion. Chromatin immunoprecipitation was performed using the Imprint Ch- IP kit followed by qRT-PCR. Human pediatric T-ALL samples were transplanted into NOD-SCID/IL2γc−/− (NSG) mice for in vitro and in vivo inhibitor studies.

Results: The RPL10 R98S mutation provided a cell survival advantage in Ba/F3 cells and in serial re-plating assays of in- BM cells derived from RPL10 R98S knock-in mice. Proteomic profiling revealed metabolic reprogramming in RPL10 R98S cells through enhanced expression of peroxisomal enzymes Acox1, Acox3 and Paox. This expression facilitated peroxisomal β-oxidation of long chain fatty acids which are substrates for PPARy and which were consequently upregulated together with CPT1A. Peroxisomal hyperactivation causes high intracellular H2O2 levels, restraining the observed elevated levels of reactive oxygen species (ROS) in RPL10 R98S cells that could not be scavenged by the increased catalase expression. High ROS levels and enhanced PPARy binding drives the constitutive overexpression of anti-apoptotic protein B-cell lymphoma 2 (Bcl-2), responsible for the leukemia cell survival benefit of RPL10 R98S cells. Bcl-2 targeted therapy using venetoclax (ABT-199) reduced the expansion of RPL10 R98S knock-in BM cells by 50%, while RPL10 WT BM cells were not inhibited by ABT-199. In vivo, DMSO or ABT-199 50mg/kg therapy was started after the engraftment of >2% human cells in the blood of mice xenografted with T-ALL samples and was maintained 1/wk till disease end stage. RPL10 R98S xenografted mice that received ABT-199 therapy presented a complete inhibition of human CD45+ leukemia progression in the blood, which was characterized by a 70-85% reduc- tion in spleen weights, and 20-50% reduction of bone marrow engraftment. Spleen weights of ABT-199 treated RPL10 R98S xenografted mice were only slightly increased as compared to the splenic weights of healthy NSG mice. In con- trast, mice xenografted with RPL10 WT T-ALL samples showed poor in vivo responses to ABT-199 treatment and all animals showed progressive disease. Bcl-2 overexpression induced by peroxisomal hyperactivation was defined as new target in RPL10 R98S defective T-ALL. Additionally, due to peroxisomal hyperactivation, a peroxisomal oxidase involved in purine degradation may have contributed to the prostate product of purine degradation, uric acid, was elevated above reference levels in the blood of RPL10 R98S mutant pediatric T-ALL patients at diagnosis (Figure 1).

Summary/Conclusions: We demonstrate that T-ALL patients have limited heterogeneity at diagnosis and that targeted single cell sequencing can be used to determine the cell of origin and the order of mutation acquisition. These data also illustrate that HSPCs at remission carry a few early, pre-leukemic events, while highly mutated HSPCs are eradicated during treatment, which is in line with long term remission in T-ALL.

Figure 1.
Summary/Conclusions: Uric acid provides an indicative biomarker of RPL10 R98S mutations in pediatric T-ALL patients, which may be used for screening, providing early diagnosis and appropriate selection of patients in whom a Bcl-2 targeted therapeutic approach could be considered.

P153

TRANSLATOME ANALYSIS OF THE T-ALL ASSOCIATED RIBOSOMAL PROTEIN L10 R98S MUTATION REVEALS ALTERED SERINE METABOLISM

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Background: We previously described a recurrent arginine-to-serine mutation on residue 98 (R98S) in ribosomal protein L10 (RPL10), with a frequency of 8.6% in pediatric T-ALL cases. The R98S mutated residue contacts the catalytic core (peptidyltransferase center, PTC) of the ribosome and causes ribosome biogenesis, Pab and Usn defects in vitro. RPL10-WT mouse lymphoid Ba/F3 cells expressing cells enhance their endogenous serine production, leaving more space for new translation. Our data suggest that the RPL10-R98S mutation may contribute to T-ALL pathogenesis by inducing translational changes.

Aims: The spectrum of translated proteins (translatome) of RPL10 R98S mutants was investigated in order to identify translational changes caused by the mutation and potentially driving oncogenicity.

Methods: We performed ribosome footprinting (RNA sequencing of ribosome bound RNA), polysomal RNA sequencing, total RNA sequencing and mass spectrometry based quantitative proteomics on engineered RPL10-R98S or RPL10-WT mouse lymphoid Ba/F3 cells.

Results: RPL10 R98S cells showed significant upregulation for 3% (n=178) of the measured proteins and a downregulation of 1% (n=68). Moreover, polyosomal RNA sequencing and ribosome footprinting showed respectively 57 and 22 genes with significantly higher translational efficiency in RPL10 R98S, and 22 and 29 genes with reduced translational efficiency. Among them, we also found genes involved in T cell differentiation and proliferation. In particular, Mapk6 presented reduced translational efficiency in the ribosome footprinting, potentially due to differences in ribosome occupancy of an upstream ORF, whereas the transcription factor Ick2, a master regulator of the upregulated transcripts, was overexpressed at the transcriptional and protein level. Interestingly, the results from the mass spectrometry and the polysomal RNA sequencing datasets showed a significant enrichment and upregulation of members of the JAK-STAT signal pathway, notably Jak1 and Ick1. Several Stats being 1-3-fold elevated at the protein level and higher translation efficiency for Lf, Ctnn, Il10r1, Gish and Ick2. Another interesting candidate showing 5-fold upregulated protein levels was phosphoserine phosphatase (Psph), a key enzyme in serine biosynthesis. Ribosome footprinting revealed that this upregulation originates from a combination of higher transcription and translational efficiency of the encoded gene. Elevated Psph protein levels were confirmed by immunoblotting in the RPL10 R98S Ba/F3 cells and in hematopoietic cell cultures derived from RPL10 R98S knock-in mice. Interestingly, harvested medium from RPL10 R98S Ba/F3 cells contained higher residual serine levels as compared to RPL10 wild type expressing cells and this medium could support the survival of wild type Ba/F3 cells. Our data suggest that RPL10 R98S expressing cells enhance their endogenous serine production, leaving more serine that can support survival of neighboring cells.

Summary/Conclusions: Analysis of the translational changes associated with the RPL10 R98S mutation reveals alterations for genes involved T cell differentiation and proliferation: the atypical MAP kinase Mapk6, whose reduced translational efficiency still needs to be validated at the protein level, and the transcription factor Ick2. Alterations were also found in the JAK-STAT signaling, an established oncogenic cascade in T-ALL. Moreover, this is the first description of a mutation in T-ALL that is linked to alterations in cellular serine biosynthesis.

P154

REPOSITIONING EXISTING DRUGS AS NOVEL THERAPEUTICS: OXIDATIVE STRESS AS A TARGET FOR HIGH-RIK LEUKAEMIA IN CHILDREN

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Background: Remarkable improvements made in the treatment of childhood acute lymphoblastic leukaemia (ALL) in past decades have resulted in 5-year survival rates approaching 90%. However, prognosis remains dismal for certain subgroups of high-risk patients, including poor responders to induction therapy, infants with ALL that harbor rearrangement of the Mixed Lineage Leukaemia (MLL/KMT2A) gene, and children with Philadelphia chromosome positive ALL. In particular, infant ALL patients with MLL disease have survival rates below 50% despite the use of intensified treatments, necessitating the development of more effective, less toxic therapeutics for them.

Aims: The aim of this study is to identify candidates that target MLL-rearranged leukaemia cells using drug-repurposing, whereby an approved drug is applied to treat a disease other than the one for which it was originally intended. This drug discovery strategy is gaining popularity as it potentially avoids the lengthy process of drug development and FDA approval.

Methods: 3707 approved drugs and pharmacologically active compounds were initially screened against an infant ALL cell line with MLL-rearrangement, PERR-485 and a paediatric leukaemia cell line wild-type for MLL, CEM, using a resazurin-based cell viability assay. Hit compounds were further tested in a panel of 10 MLL-rearranged ALL and 10 leukemia cell lines. Compounds were subsequently evaluated in vitro for cytotoxic activity against a panel of 20 paediatric high-risk ALL patient-derived xenograft (PDX) cells. Apoptosis was measured by Annexin V positivity and PARP cleavage. Reactive oxygen species (ROS) levels were assessed by DCF-DA staining and detection by flow cytometry. Nrf2 protein expression levels were measured by Western blotting.

Results: The screen resulted in the identification of two FDA-approved drugs that were preferentially cytotoxic against MLL-rearranged ALL and other leukaemia cell lines, compared to solid tumours and normal cells. Auranofin was discovered developed for rheumatoid arthritis and was later fast-tracked into Phase II clinical trial for adult chronic lymphocytic leukaemia, while Disulfiram, which was developed for treatment of chronic alcoholism, is currently in several clinical trials for cancers including metastatic melanoma and glioblastoma. These drugs also showed potent activity in high-risk paediatric leukaemia PDX cells cultured in vitro, including MLL-rearranged ALL and Philadelphia-positive ALL with IC50 values between 100-400 nM for Auranofin and 30-60 nM for Disulfiram. Induction of apoptosis was evident at 6 hours post Auranofin treatment, or after 12 hours Disulfiram treatment. Each drug significantly increased intracellular ROS as early as one hour post-treatment (p<0.01), which was accompanied by induction of Nrf2, a master regulator of the antioxidant response. Incubation with ROS scavenger N-acetyl cysteine prior to treatment with either drug prevented the increase in cellular ROS levels (p<0.05) and rescued cells from apoptosis (p<0.0001), indicating involvement of reduction-oxidation and increased ROS generation as mechanisms of leukaemia cell death induced by these drugs.

Summary/Conclusions: In summary, we have identified two FDA-approved drugs that demonstrated potent anti-leukaemia activity through induction of ROS, potentially opening up new avenues for clinical treatment of high-risk paediatric ALL. We will now be testing these potential therapeutics in vivo using relevant PDX models of high-risk paediatric ALL.

P155

TP53 MUTATIONS DISRUPTING DNA BINDING LEAD TO CHEMOTHERAPY RESISTANCE IN ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background: Polychemotherapy resistance is a major challenge in the treatment of children with relapsed acute lymphoblastic leukemia (ALL). Mutation of TP53 is tightly associated with poor response to treatment in ALL relapse patients.

Aims: We studied mutations of TP53 in ALL relapses and in six ALL cell lines to shed light on mechanisms and pathways mediating TP53 dependent drug resistance in relapsed ALL. First, we analyzed the spectrum of TP53 mutations in ALL relapses and correlated it to treatment response of patients. Second, we studied drug sensitivity in TP53 wild type (wt) versus TP53 mutant ALL cell lines.

Methods: We performed whole genome sequencing of 3707 approved drugs and pharmacologically active compounds were screened against an infant ALL cell line with MLL-rearrangement, PERR-485 and a paediatric leukaemia cell line wild-type for MLL, CEM, using a resazurin-based cell viability assay. Hit compounds were further tested in a panel of 10 MLL-rearranged ALL and 10 leukemia cell lines. Compounds were subsequently evaluated in vitro for cytotoxic activity against a panel of 20 paediatric high-risk ALL patient-derived xenograft (PDX) cells. Apoptosis was measured by Annexin V positivity and PARP cleavage. Reactive oxygen species (ROS) levels were assessed by DCF-DA staining and detection by flow cytometry. Nrf2 protein expression levels were measured by Western blotting.

Results: The screen resulted in the identification of two FDA-approved drugs that were preferentially cytotoxic against MLL-rearranged ALL and other leukaemia cell lines, compared to solid tumours and normal cells. Auranofin was discovered developed for rheumatoid arthritis and was later fast-tracked into Phase II clinical trial for adult chronic lymphocytic leukaemia, while Disulfiram, which was developed for treatment of chronic alcoholism, is currently in several clinical trials for cancers including metastatic melanoma and glioblastoma. These drugs also showed potent activity in high-risk paediatric leukaemia PDX cells cultured in vitro, including MLL-rearranged ALL and Philadelphia-positive ALL with IC50 values between 100-400 nM for Auranofin and 30-60 nM for Disulfiram. Induction of apoptosis was evident at 6 hours post Auranofin treatment, or after 12 hours Disulfiram treatment. Each drug significantly increased intracellular ROS as early as one hour post-treatment (p<0.01), which was accompanied by induction of Nrf2, a master regulator of the antioxidant response. Incubation with ROS scavenger N-acetyl cysteine prior to treatment with either drug prevented the increase in cellular ROS levels (p<0.05) and rescued cells from apoptosis (p<0.0001), indicating involvement of reduction-oxidation and increased ROS generation as mechanisms of leukaemia cell death induced by these drugs.

Summary/Conclusions: In summary, we have identified two FDA-approved drugs that demonstrated potent anti-leukaemia activity through induction of ROS, potentially opening up new avenues for clinical treatment of high-risk paediatric ALL. We will now be testing these potential therapeutics in vivo using relevant PDX models of high-risk paediatric ALL.
GENETIC ACTIVATION AND THERAPEUTIC TARGETING OF PIM1 IN T-CELL ACUTE LYMPHOBLASTIC LYMPHOMA

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Background: T-cell acute lymphoblastic leukemia (T-ALL) and T-cell acute lymphoblastic lymphoma (T-LBL) are aggressive immature T-cell malignancies that are considered one disease entity according to the World Health Organization (WHO). Both T-ALL and T-LBL are often characterized by immunoproliferations leading to aberrant activation of proto-oncogenes.

Aims: Despite some genetic and phenotypic similarities between T-TALL and T-LBL, T-ALL risk group stratification cannot be extrapolated to T-LBL patients. Therefore, it is our goal to find new T-LBL markers and develop new targeted therapies based on those T-LBL specific markers.

Methods: We used an IL-7-dependent leukemia T-cell line (TAIL7) and “primary” cells from patient-derived xenografts (PDX). We used inhibitors of PI3K (LY294002), mTOR (rapamycin), MEK1/2 (UO126) and ULK1/2 (MRT68921). Analysis of viability and cell size was performed by flow cytometry. Signaling pathway activation and LC3-I/-II conversion was performed by western blot analysis. LC3 puncta formation was assessed by confocal microscopy. Autophago-some/autolysosome formation was analyzed by electron microscopy.

Results: We show that in optimal culture conditions (medium with serum) IL-7 inhibits autophagy in T-LBL, albeit in a complex manner that involves triggering both pro- (via MEK/Erk) and anti- (via PI3K/Akt/mTOR) autophagic signaling. In this scenario, IL-7-mediated viability relies on the latter pathway, as we previously described. In contrast, under stress conditions (serum starvation) IL-7 promotes autophagy in leukemia cells. In this situation, IL-7-mediated survival partially relies on autophagy activation and strictly requires MEK/Erk activation. Mechanistically, we provide evidence that depending on culture conditions, IL-7 can balance the relative activation of PI3K/Akt/mTOR and MEK/Erk pathways towards or against autophagy in order to consistently promote T-LBL cell viability.

Summary/Conclusions: Our results suggest that IL-7 makes use of a ‘flexible strategy’ to promote T-LBL cell viability by recruiting both pro- and anti-autophagy pathways, with the potential to activate autophagy or recruit pro-tumor cell death depending on the microenvironmental conditions. Our data strengthen the notion that combination therapies against PI3K/Akt/mTOR and MEK/Erk pathways may be of particular relevance in the context of T-LBL.

PRECLINICAL ACTIVITY OF ENTOSPLETINIB IN CHILDHOOD B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: T-cell acute lymphoblastic leukemia (T-ALL) constitutes an aggressive subset of ALL, the most frequent childhood malignancy. T-ALL cases are high risk and a significant fraction of the patients still relapse despite intensive chemotherapy, prompting the need for a deeper understanding of T-ALL biology in order to develop novel therapies. Autophagy is a homeostatic intracellular process characterized by the sequestration of cytoplasmic compartments within double-membrane vesicles (autophagosomes) to promote their degradation. Importantly, autophagy is upregulated during starvation, cellular stress or in rapidly dividing cells, such as cancer cells, as a compensatory mechanism to provide nutrients and stress relief. By mitigating stress and allowing cell survival, autophagy may serve as a pro-tumoral mechanism. On the other hand, persistent autophagy can lead to cell death and thereby prevent tumor growth. Interleukin-7 (IL-7) is essential for normal T-cell development but there is considerable evidence that IL-7-mediated signaling can also contribute to leukemogenesis. A majority of T-ALL patients expresses the IL-7 receptor and IL-7 accelerates T-ALL disease progression in vivo and promotes T-ALL cell proliferation, survival and metabolic activation in vitro via PI3K/Akt/mTOR pathway (a master negative regulator of autophagy). IL-7 can also activate MEK/Erk pathway (which has been implicated in promotion of autophagy).

Aims: Since IL-7 has the ability to activate signaling pathways with potentially opposing roles in autophagy regulation, our goal was to explore the actual impact of IL-7 on the autophagic process in T-ALL cells and elucidate its molecular mechanisms and functional consequences.

Methods: We used an IL-7-dependent leukemia T-cell line (TAIL7) and “primary” cells from patient-derived xenografts (PDX). We used inhibitors of PI3K (LY294002), mTOR (rapamycin), MEK1/2 (UO126) and ULK1/2 (MRT68921). Analysis of viability and cell size was performed by flow cytometry. Signaling pathway activation and LC3-I/-II conversion was performed by western blot analysis. LC3 puncta formation was assessed by confocal microscopy. Autophago-some/autolysosome formation was analyzed by electron microscopy.

Results: We show that in optimal culture conditions (medium with serum) IL-7 inhibits autophagy in T-ALL, albeit in a complex manner that involves triggering both pro- (via MEK/Erk) and anti- (via PI3K/Akt/mTOR) autophagic signaling. In this scenario, IL-7-mediated viability relies on the latter pathway, as we previously described. In contrast, under stress conditions (serum starvation) IL-7 promotes autophagy in leukemia cells. In this situation, IL-7-mediated survival partially relies on autophagy activation and strictly requires MEK/Erk activation. Mechanistically, we provide evidence that depending on culture conditions, IL-7 can balance the relative activation of PI3K/Akt/mTOR and MEK/Erk pathways towards or against autophagy in order to consistently promote T-LBL cell viability.

Summary/Conclusions: Our results suggest that IL-7 makes use of a ‘flexible strategy’ to promote T-LBL cell viability by recruiting both pro- and anti-autophagy pathways, with the potential to activate autophagy or recruit pro-tumor cell death depending on the microenvironmental conditions. Our data strengthen the notion that combination therapies against PI3K/Akt/mTOR and MEK/Erk pathways may be of particular relevance in the context of T-LBL.
Background: B-cell acute lymphoblastic leukemia (B-ALL) is the most common malignancy of childhood and is highly curable with modern risk-adapted chemotherapy. However, 15-20% of children and >60% of adults with B-ALL develop chemoresistance and relapse, indicating need for new therapies. Addition of kinase inhibitors to chemotherapy for patients with BCR-ABL1-rearranged (Ph+) B-ALL has dramatically improved event-free and overall survival, and similar approaches are now under active clinical investigation in patients with BCR-ABL1-like (Philadelphia chromosome-like or Ph-like) B-ALL. Recent studies have demonstrated activated spleen tyrosine kinase (SYK) signaling in various genetic subtypes of B-ALL and preclinical activity of the SYK/FLT3/JAK inhibitor fostamatinib. However, SYK inhibition in B-ALL and potential correlation with specific leukemia-associated mutations remains incompletely characterized. We hypothesized that constitutive activation of SYK signaling occurs across a genetic spectrum of infant and high-risk childhood B-ALL and can be therapeutically targeted in vivo with the selective SYK inhibitor entospletinib (ento).

Aims: (1) Assess basal SYK signaling activation in childhood B-ALL specimens. (2) Quantify treatment efficacy, pharmacokinetics (PK), and pharmacodynamic (PD) effects of ento in childhood B-ALL patient-derived xenograft (PDX) models.

Methods: Total and phosphorylated (p) SYK levels were assessed by Simple Western analysis of splenic lysates from NSG mice well-engrafted with primary pediatric B-ALL specimens (n=19 Ph-like, n=4 infant KMT2A-rearranged (R), and n=4 infant non-KMT2A-R PDX models) to identify leukemias with constitutive SYK signaling activation. To assess in vivo activity of SYK inhibition, selected B-ALL PDX models with high basal pSYK (n=2) were treated with continuous provided control 0.03% or ento 0.07% chow. Cohorts of SYKtarget phosphoproteins were more pronounced in 0.07% ento-treated animals. Without alterations in total SYK protein levels. In general, PD inhibition of SYK signaling occurred after 21-28 days, and peripheral blood and spleens were harvested for downstream studies. Flow cytometric analyses of murine tissues were performed to assess initial human ALL engraftment and to measure ento treatment responses. PK and PD assessments were performed in terminal peripheral blood and spleens, respectively.

Results: Constitutive pSYK signaling was observed in 10/19 Ph-like, 4/4 KMT2A-R, and 1/4 non-KMT2A-R B-ALL specimens. Ento treatment of KMT2A-MLLT3 (ALL3103) and Ph-like NUP214-ABL1 (NH011) PDX models significantly inhibited ALL proliferation in vivo versus control animals at both 0.03% and 0.07% chow formulations (representative data in Figure 1; p<0.05). Steady state concentrations were maintained throughout the study duration with terminal PK values of 3.3 (± 0.5) and 7.9 (± 1.0) μM (0.03% and 0.07% ento arms, respectively). PD studies demonstrated dose-dependent in vivo inhibition of pERK measured in human leukemia cells within spleens of ento-treated mice (Figure 1). PK values were was observed in various trials for some of these inhibitors (mAbs, GSIs), but treatment and exposure were usually limited due to toxicities, mainly related to gastro-intestinal adverse events. On the contrary, in human cancers harbouring NOTCH gene fusion due to chromosomal translocations or specific NOTCH mutations, the use of mAbs and GSIs will have very limited clinical benefits. Cellestia has decided to follow a disruptive approach, by blocking NOTCH signalling in the most downstream part of the NOTCH cascade, at the level of the NOTCH transcriptional activation complex, using small molecule inhibitors.

Methods: Here we report the pharmacological characterization of CB-103, a first-in-class orally-active small molecule inhibitor of the NOTCH transcriptional activation complex.

Results: We demonstrate that in vitro CB-103 potently inhibits NOTCH signalling in various leukemic and lymphoma cell lines, and T-ALL blasts derived from relapse/refractory patients. In addition, CB-103 exhibited anti-tumor efficacy in multiple in vivo models of NOTCH-driven T-ALL using T-ALL cell lines and patients derived xenograft models.

Summary/Conclusions: Toxicology studies have been completed and clinical development of CB-103 with a first-in-human Phase I/IIA clinical study in advanced solid tumors and haematological malignancies is under preparation.
Acute lymphoblastic leukemia - Clinical 1

IKZF1Δ4-7 CAN BE EASILY SCREENED BY PCR BUT DOES NOT PRE-DICT OUTCOME IN ADULTS WITH ACUTE LYMPHOBLASTIC LEUKAEMIA; DATA FROM 490 PATIENTS ENROLLED ON THE UKALL14 TRIAL


LEUKAEMIA; DATA FROM 490 PATIENTS ENROLLED ON THE UKALL14 ISRCTN 66541317

IKZF1Δ4-7 and to determine its utility as a prognostic marker in B precursor ALL using data from UKALL14 (ISRCTN 66541317) - a multicentre phase 3 randomised trial for adults aged 25-65 years.

Methods: Diagnostic DNA from 490 bone marrow samples was screened for IKZF1Δ4-7 using an endpoint PCR assay using primers located in introns 3 and 7. The lower limit of detection was determined by serial dilution of DNA from the iKE6-expressing cell line SUP-B15 and calculated to be 0.001%. A total of 95 samples were also tested using the MLPA P335 kit to detect the full spectrum of IKZF1 deletion. Sanger sequencing confirmed the breakpoints in 27 cases.

Results: The median age of the patients tested was 46 years (range 25-65). Overall IKZF1Δ4-7 was detected in 97/490 (20%) patients but the frequency varied by genetic subtype. Patients with BCR-ABL1 fusion had the highest IKZF1Δ4-7 frequency (46/150, 31%) followed by patients with B-other ALL (29/154, 19%). Patients with other classic cytogenetic abnormalities harboured significantly fewer IKZF1Δ4-7 – low hypodiploidy (3/26), MLL gene fusions (3/31), t(1;19), (1/11), high hyperdiploidy (2/9) and iAMP21 (0/3). MLPA did not detect any IKZF1Δ4-7 deletions that were not detected by PCR but did identify several samples with alternative IKZF1 deletions affecting different exons (see Table 1). By contrast, the PCR assay did detect six IKZF1Δ4-7 deletions undetected by MLPA, consistent with the higher sensitivity of this approach. Interestingly, three of these three samples harboured alternative IKZF1 deletions in addition to IKZF1Δ4-7. In 70 (14%) cases, we observed a "faint" PCR band. Since the biological relevance of this was not clear, the 'faint' bands were not included in the final analysis. Interestingly the frequency of these "faint" bands was similar across all genetic subtypes: BCR-ABL1 (14%), B-other (15%), MLL (21%), low hypodiploidy (19%). We examined the impact of IKZF1Δ4-7 on achievement of CR, persistence of minimal residual disease (MRD) did not persist after phase 1. We did not identify any association between IKZF1Δ4-7 and any of the other outcome parameters tested.

Table 1.

Summary/Conclusions: IKZF1Δ4-7 can be detected by a simple and cheap PCR assay, which is more sensitive than MLPA. The frequency of IKZF1Δ4-7 was broadly comparable with previous studies. However, we did not find an association between IKZF1Δ4-7 and clinical outcome in the large clinical trial sample set. We are in the process of evaluating the impact of other IKZF1 lesions.

P161

PROGNOSTIC SIGNIFICANCE OF MINIMAL RESIDUAL DISEASE DETECTED BY MLL FUSION GENE TRANSCRIPTS IN INFANT ACUTE LYMPHOBLASTIC LEUKAEMIA, UPDATED RESULTS OF 76 PATIENTS ENROLLED INTO MLL-BABY STUDY

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Background: Fusion gene transcripts (FGTs) are rarely used for minimal resid-ual disease (MRD) monitoring in acute lymphoblastic leukemia (ALL) cases, except of Ph-positive ALL. However in infant ALL, where MLL gene rearrange-ments are found the majority of cases, MLL FGTs are attractive targets for MRD detection.

Aims: To estimate prognostic significance of MLL by qualitative detection of different MLL FGTs in infant ALL treated by MLL-Baby protocol.

Methods: Seventy six infants (27 boys and 49 girls) with median age of 5.8 months (range 0.3-11.83) were included in the current study. Among them were 39 (51.3%) MLL-4F4-positive cases, 14 (18.4%) MLL-MLLT1-pos-itive, 12 (15.8%) MLL-MLLT3-positive, 6 (7.9%) MLL-MLT10-positive, 4 (5.3%) MLL-EP515-positive. MDR detection was performed in BM samples by real-time quantitative PCR and nested RT-PCR with sensitivity non-less than 1.0E-04. MRD-negativity was defined as absence of FGTs in the both assays. Median of follow-up period in the observed group was 6.4 months. Informal consent was obtained in all cases.

Results: We confirmed our earlier finding that the most informative TP for the MLL-baby protocol was TP4 as MRD negativity by qualitative detection at TP4 led to unfavorable outcome in both MLL-4F4-positive patients stratified to high-risk arm of MLL-Baby protocol (EFS 0.05±0.04 vs 0.07±0.08 p<0.0001; cumulative incidence of relapse 0.78±0.10 vs 1.12±0.07 p<0.001, respectively) and for all others MLL-rearranged patients treated by intermediate risk (imR) arm (EFS 0.00 vs 0.07±0.11 p<0.0001; cumulative incidence of relapse 1.0 vs 0.29±0.10 p<0.0001, respectively). There were no significant differences in initial patients' characteristics and treatment response criteria (on days 8, 15, 36) among 38 MRL-positive and 38 MRL-negative patients. Multivariate analysis revealed that initial CNS disease (hazard ratio (HR) 2.703, 95% CI 1.255-5.284, p=0.011), m1 status of BM on day 15 (HR 3.909, 95% CI 1.465-10.615, p=0.003) and MRD-negativity at TP4 (HR 6.950 95% CI 2.617-18.456) were significant covariates with negative impact on hazard of unfavorable event. Based on dismal outcome of MRL-positive imR patients we tried to augment their ther-a py and relocated 5 of them from imR group to HR arm after TP4. Although all 5 subsequently relapsed, we also wanted to find out which characteristics might predict relapse in imR patients who were MRL-negative at TP4 (n=5). Of all, these 5 relapsed patients (100%) had initial CNS disease while CNS disease was detected only in 2 out of 19 imR patients (10.5%) who stayed in complete hematological and molecular remission (p=0.003). Also all 5 relapsed imR patients who were MRL-negative at TP4 had breakpoint positions within intron 11 of MLL gene and they were MRL-positive by flow cytometry (MRL ≥0.1%) on day 15. None of MRL-negative patients by flow cytometry (MRL <0.1%) on day 15 relapsed later on (p<0.001).

Summary/Conclusions: MRD monitoring by detection of MLL FGTs was fea-sible and had significant prognostic impact. MRD-negativity at TP4 was an inde-pendent factor of unfavorable outcome in infants with MLL-rearranged ALL enrolled into MLL-Baby protocol irrespective of treatment arm. Treatment inten-sification for MRL-positive at TP4 in imR patients did not improve their outcome. MRD-negativity at TP4 in imR group was associated with MRD-negativity by flow cytometry on day 15, MLL breakpoint positions within intron 11 gene and initial CNS disease.

P162

PRO-T CELL ALL/LBL: AN ULTRA-HIGH RISK CD2-NEGATIVE DISEASE SUBTYPE IN ADULTS DEFINED BY FLOW CYTOMETRY

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Background: Risk factors for T-LBL have not been systematically evaluated, in contrast to T-ALL.

Aims: Our aim was to define immunophenotype of T-LBL/ALL in 71 consecutive patients by use of the flow cytometry (FCM) of tissue aspirates if peripheral blood (PB) and bone marrow (BM) were uninvolved. We also evaluated prognostic value of immunophenotype according to WHO 2008 subtype and ETP (Early T-Cell Phenotype) definition in adult patients with T-LBL/ALL treated on uniform ALL protocol.

Methods: Between 1997 and 2015, 71 adult patients with T-LBL/ALL were treated according to the GMAIL 05/93 and T-LBL/2004 protocols. Immunophenotype was determined by immunohistochemical staining and by FCM of cellular suspension obtained from lymph nodes (n=31), mediastinal mass (n=12) or nasopharyngeal/perimandibular infiltration (n=2) by fine needle aspiration biopsy (FNAB), as well as of BM (n=10), PB (n=7) and pleural fluid (n=9). Disease subtype was defined according to WHO 2008 classification: pre-B (CD10-), pre-T (CD2+), common CD1a+, residual/mature (scCD3+). Recognition of pan-T cell CD antigen (pTag) expression included: CD1a, CD2, scCD3, CD4, CD5, CD7. ETP-TALL/ALL immunophenotype was defined as follows: absent (up to 5% positive cells) CD1a and CD8 expression, absent or dim (75% positive cells) CD5, expression (25% positive cells) of 1 or more myeloid (CD13/CD33/CD15) or stem cell (CD34, HLA-DR) markers.

Results: Patient characteristics: ALL (BM+ > 20%): n=26(37%); LBL: n=45(63%); BM+<20% involvement (LB): 27%; age: 35 years; 72%: males: 67%: mediastinal mass (MM): 92%; primary CNS+: 8%; Immunophenotype: pro-T: 21%; pre-T: 17%; common 55%; residual/mature 10%; of pTag present: 0-3: n=25(36%) or 4-7: n=45(64%) of pts. Most frequently expressed pTag were: CD7: 97%; CD5: 87%; CD7: 74%, CD1a: 58%. Myeloid markers: CD13/33/15 were expressed in 13%/26%/10% and stem cell markers: CD34/CD5 were expressed in 46%/48% of pts with SR, 40%/31% of pts with HR/very HR features such as pro-B phenotype (n=11: 27.2%, 54.5%), early-T phenotype (n=12: 41.6%, 50%) and high-risk (HR) features defined by presence of any one positive (n=19), 43%; both positive (n=23); 44% (P<0.001). Five-year OS (95%CI) follow up of 137 (0.99, 1.733) months, 5-yr OS and DFS (95%CI) was 64.4% and 35.6% had B- and T-ALL, respectively, 44.4% were SR, 10% HR.

Summary/Conclusions: Survival of T-LBL/ALL pts depends on CD1a and CD2 expression as well as on WHO subtype. ETP is a non-uniform category by pro-17/Pre-T-cell origin. ETP phenotype was non-significant factor for OS/DFS (P=0.10/0.17) unless consistent with pro-T cytotype (CD2-), only 19% pts alive. Pro-T (CD2-) is an ultra-high risk subtype of T-LBL/ALL and novel treatments are needed to improve pts outcomes.
Background: The outcome for older adults with acute lymphoblastic leukaemia (ALL) is unsatisfactory. The UKALL12/ECOG2993 study showed that high risk cytogenetic abnormalities, were common, as well as lower rates of complete remission (CR) and 5 year overall survival (OS) in those aged 55–65 years of age as compared to younger persons. There are few studies which focus on older patients with ALL, despite an increasing incidence with age.

Aims: A trial to establish an age-appropriate baseline chemotherapy from which to design widely-applicable studies of novel agents in older people with ALL.

Methods: UKALL66+ offers five ‘Arms’ to be decided by investigator and patient choice: Arm A= Philadelphia chromosome positive (Ph+), Arm B= Non-intensive (designed to be delivered primarily out of hospital), Arm C= Intensive, Arm D= Intensive+ and Arm E= Registration only (in which treatment is at investigators discretion, including no active therapy). Any elderly patient with newly diagnosed ALL is eligible. There are no exclusions for co-morbidities, including prior malignancies. Baseline characteristics of each group including Charlon index, ECOG, Karnofsky and CRASH scores are being collected. The primary endpoint is the rate of complete remission (CR) after 2 phases of induction. Secondary objectives include determination of MRD status at 3 time points, EFS and OS at 1 year, treatment related mortality and quality of life.

Results:

The incidence of ALL in older patients is challenging to treat, with a difficult balance between efficacy and toxicity. We observed a high rate of high risk cytogenetics (57%) patients who received palliative therapy only, which included TKIs, chemotherapy, or hospice. The other 53 (84%) received induction chemotherapy. Only 12 (23%) had an up-front dose reduction due to comorbidities. 32 (60%) received Hyper-CVAD, concomitantly with rituximab in 11 (34%) pts. & TKIs in 9 (28%) pts. 21 (40%) pts received other regimens, of which 14 (67%) had asparaginase-based chemotherapy. Only 2 (4%) pts who received induction chemotherapy died within the first 60 days; both received Hyper-CVAD. Median number of cycles to achieve CR was 1 (1-8) with CR/CRi rate of 93%, & median time to CR1 was 34 days (19-459). 3 pts who underwent palliative chemotherapy achieved CR (all had Ph+- disease & received TKIs). 7 pts (13%) had primary induction failure. 50% of pts relapsed within a median time of 12.6 (3.6-72.8) months. Only 10 pts underwent allogeneic hematopoietic stem cell transplantation (HSCT), of which 2 (20%) relapsed in less than 180 days. Median survival after HSCT was not reached. Predictors of survival: Elderly ALL has worse mOS compared to our adult ALL cohort, 17.2 (IOR: 11.7-32.9) vs 52.1 (IOR: 27.6-169.9) mon (p=0.0016). In a univariate analysis model which included multiple variables, only ECOG PS ≥2, WBC>30,000, CDKN2A del, & CNS leukaemia were statistically significant, however only CNS leukemia (p=0.0009) & WBC (p=0.0168) retained their statistical significance in multivariate mode, with a trend in CDKN2A del (p=0.06) (Figure 1).

Summary/Conclusions: ALL in older patients is challenging to treat, with a difficult balance between efficacy and toxicity. We observed a high rate of high risk cytogenetics, especially notable being the low hypodiploidy. Initial high CR rates are seen in those with Ph+ve disease, this does not appear to translate into improved PFS and OS when compared with Philadelphia negative disease. The commonest cause of death in this group is ALL. We will use our baseline data to develop appropriate regimens for future studies of novel agents.
Background: The outcome of adults and elderly (>60 years) patients with Ph+ ALL has improved since the introduction of tyrosine kinase inhibitors (TKI), used alone or in combination with chemotherapy during induction. Before 2005, all these patients were treated with chemotherapy; from 2005, a TKI-based “chemo-free” induction strategy was applied.

Aims: To evaluate the outcome of patients followed from 1996 at a single Center, and to correlate the short- and long-term responses with: a) induction treatment (chemotherapy or TKI); b) age; c) TKI used (imatinib or dasatinib); d) fusion protein; e) allogeneic stem cell transplant (SCT).

Methods: Sixty-eight patients (29M/39F) were treated; median age was 50 years (20-88) and 16 were elderly patients; 43 cases had the p190 protein, 19 the p210 and 6 had both; the latter 2 groups were merged together for further analyses. Fifty-two patients were enrolled in clinical trials. Median follow-up is 105 months (13-224).

Results: As induction, 28 patients received chemotherapy, 2 chemotherapy+TKI (considered as “chemotherapy+TKI group”) and 38 TKI alone (24 imatinib and 14 dasatinib). All cases received TKI during consolidation/maintenance when it became available. All elderly patients but 1 received a TKI alone (plus steroids). Upon induction, 44 patients received consolidation chemotherapy, including 5 elderly. A SCT - carried out virtually only in adults - was performed in first complete remission (CR) in 13 cases (5 in the chemotherapy+TKI and 8 in the TKI groups). Overall, 91% patients achieved a CR, OS and DFS at 100 months are 42% and 45.5%, respectively. Among the 30 patients in the chemotherapy+TKI group, 25 (83%) achieved a CR, 4 were refractory and 1 died in induction; in the TKI group (n=38), 37 (97%) achieved a CR and 1 was refractory. Differences are statistically significant (p=0.03). Refractoriness was more frequent in p210+ than in p190+ cases (12% vs 5%); this finding did not translate into significantly different OS and DFS (30% vs 48% and 32% vs 51%, respectively). When patients were stratified by age, adults had a significantly better OS and DFS at 100 months than elderly (53% vs 19%, p=0.04, and 57% vs 20%, p=0.03, respectively), even more marked in multivariate analysis (HR=2.4; p=0.017, respectively). The TKI used (imatinib or dasatinib) did not impact in adults, while a significant advantage in OS and DFS was observed for elderly patients receiving dasatinib (Figure 1: this might be due to the greater activity of dasatinib and also highlights the importance of consolidation chemotherapy, performed almost exclusively in adults. Considering adults only, within the chemotherapy+TKI group, 5 patients were transplanted and 19 were not: all transplanted cases are in CR, while in the non-transplanted cases 6 are in CR, 11 have relapsed and 2 have died in CR (p=0.01); within the TKI group, 8 patients underwent a SCT and 15 did not; of the transplanted cases, 6 are in CR and 2 have died due to complications, while 11 of the non-transplanted patients are in first CR, 3 have relapsed and 1 has died in first CR (p=n.s.). Of the 5 patients transplanted in second CR, only 1 is alive.

Figure 1.

Summary/Conclusions: This study further underlines the benefit of an induction based on a TKI alone. Since age holds strong prognostic significance, our results suggest that while chemotherapy followed by consolidation chemotherapy is the optimal choice for adults, in elderly cases dasatinib is more appropriate, since patients are often unfit to receive further chemotherapy. Finally, the advantage of SCT needs to be carefully redefined in the TKI era.
Results: Our series included 69 boys and 57 girls diagnosed with acute leukemia, with a median age of 6.1 years (range 0-17.4 years). We included 12 infant patients (<1 year old). Eighty-two (65%) patients had B-cell precursor acute lymphoblastic leukemia (BCP-ALL), 24 patients T-cell ALL and 20 patients had acute myeloblastic leukemia (AML). Globally, we found higher expression levels of class I HDAC isozymes (HDAC 1, 2, 3 & 8) in leukemic samples as compared to non-neoplastic samples, as previously reported. Interestingly, some HDAC isoforms associated with specific genetic aberrations. Those patients with rearrangement of MLL (KMT2A) gene (n=18, including 9 BCP-ALL and 9 AML; 7 infants and 11 pediatric) showed a significantly higher expression of HDAC9 (p<0.0001) and a statistically significant underexpression of HDAC1 and HDAC3 (p=0.003 & p=0.02, respectively, see Figure 1). Infants (n=12) had also a significantly lower expression of HDAC7 (p=0.043). In the same line, all pediatric patients with pro-B phenotype (CD10 negative) had low levels of HDAC7, but differences did not reach a statistical significance. After a median follow-up of 5.9 years, 15 patients died, with an overall survival (OS) of 50% at 5 years. OS rates for T-ALL and AML patients were 55% and 50%, respectively (Fig. 1). In the BCP-ALL subgroup, the expression of HDACs did not predict outcome, and only CNS infiltration and leukocytosis were unfavorable risk factors for OS. Again, CNS+, high WBC count and presence of minimal residual disease (MRD) post-induction were predictive for worse event free survival (EFS). Although the number of cases is low and these results must be taken with caution, T-ALL patients with the highest expression of HDAC3 (upper quartile) significantly correlated with worse OS (94% vs 25%, p=0.001) and a trend towards worse EFS (89% vs 53%, p=0.06). The only significant risk factor for EFS in this subgroup was the presence of MRD after induction (p=0.003).

P169
MINIMAL DISSEMINATED DISEASE DETECTION BY FLOWCYTOMETRIC IMMUNOPHENOTYPING IN T-CELL ACUTE LYMPHOBLASTIC LYMPHOMA
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Background: T-cell acute lymphoblastic lymphoma (T-LBL) with minimal disseminated disease (MDD) is defined as the presence of T-LBL with <25% blasts in the peripheral blood (PB) and/or bone marrow (BM) by morphology and the presence of immunophenotypically abnormal T-lymphoblasts in bone marrow by flowcytometry. Published literature regarding the prevalence and clinical significance of this rare subgroup is sparse. In this study we analysed the prevalence of minimal disseminated disease in cases of T-LBL with <25% blasts identified by morphology and the presence of immunophenotypically abnormal T-lymphoblasts in bone marrow by flowcytometry. We report here the prevalence and clinical significance of minimal disseminated disease in T-LBL.

Aims: To evaluate the prevalence of minimal disseminated disease in bone marrow in cases of T-cell acute lymphoblastic lymphoma with <25% blasts in PB and BM using 8-10 colour flowcytometric immunophenotyping and evaluate the clinical and immunophenotypic features.

Methods: This is a retrospective analysis of 42 cases of T-LBL with predominantly lymphomatous presentation with <25% blasts in peripheral and bone marrow. The following parameters were taken into account including complete hemogram, peripheral blood examination, bone marrow morphology and immunophenotyping, CSF analysis, pleural fluid morphology and immunophenotyping, tissue biopsy (lymph node or mediastinal mass), PET-CT findings and LDH levels. Flowcytometric immunophenotyping on bone marrow was performed on a 3 laser 10 color Beckman-Coulter Navios® platform and analysed using Kaluza® software. A minimum of 1,000,000 events were acquired and the presence of minimal disseminated disease was noted.

Results: A retrospective analysis of 42 cases of T-LBL with <25% blasts in peripheral and bone marrow was done. The mean age was 12.2 years (Range:2-48 years). M:F ratio was 1:1.7. Nearly all patients had normal haemoglobin, total leucocyte count and platelet counts. LDH was raised in majority of the patients (Mean 674 U/L; N=190 U/L). CSF examination was negative in all cases indicating that it is unlikely to have CNS involvement in patients with <25% blasts in PB and BM. Minimal disseminated disease was seen in 12 cases (12/42=28.6%) of cases. Of the 12 cases with minimal disseminated disease two cases were near early T-cell precursor acute lymphoblastic leukemia (near ETP-ALL) type and none were of ETP-ALL type. None of the cases showed circulating blasts in PB. The mean (range) bone marrow blast count in the group without MDD was 2.4% (0-4%) and in the group with MDD was 5.1% (0-15%). In the group with MDD (12 cases), only 5 cases showed >5% blasts/hematogones identifiable by morpho- logy. This indicates flowcytometry is necessary in cases with <5% blasts to pick up cases of MDD. PET-CT was not sensitive to pick-up MDD as increased FDG uptake was seen in only a single case of MDD; it was negative in all cases without MDD. MDD by flowcytometry ranged from 0.007% to 18.5% (mean: 3.6%; median: 4%) (Figure 1).

P170
INOTUZUMAB OZOGAMICIN IN COMBINATION WITH LOW-INTENSITY CHEMOTHERAPY (MINI-HYPER-CVD) AS FRONTAL LINE THERAPY FOR OLDER PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA: UPDATED RESULTS FROM A PHASE III STUDY
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Background: Older patients (pts) with acute lymphoblastic leukemia (ALL) have poor tolerance of intensive chemotherapy, and novel strategies are needed in this population. In pts with relapsed/refractory ALL, inotuzumab ozogamicin (InO), an anti-CD22 antibody-drug conjugate, has been shown to improve survival compared to salvage chemotherapy.

Aims: We designed a phase III trial to evaluate the safety and efficacy of low-intensity chemotherapy (mini-Hyper-CVD) plus InO as frontline treatment for older pts with newly diagnosed ALL.

Methods: Pts ≥60 years of age with newly diagnosed Philadelphia chromosome-negative pre-B received mini-Hyper-CVD (compared to hyper-CVAD: no anthracycline, 50% dose reductions of cyclophosphamide and dexamethasone, 75% dose reduction of methotrexate, 85% dose reduction of cytarabine). InO was given on day 3 of the first 4 cycles. The first 6 pts received InO at a dose of 1.3 mg/m² for cycle 1 followed by 0.8 mg/m² for cycles 2-4; pts 7-34 received 1.8 mg/m² for cycle 1 followed by 1.3 mg/m² for cycles 2-4. Due to concern for veno-occlusive disease (VOD), the protocol was amended so that pts 35+ received InO at a dose of 1.3 mg/m² for cycle 1 followed by 1.0 mg/m² for cycles 2-4. Rituximab was given during the first 4 cycles in pts with CD20 expression ≥20%; all pts received IT chemotherapy prophylaxis with the first 4 cycles. Pts in CR after 8 cycles then received POMP maintenance for up to 3 years.
Results: Between 4/2012 and 12/2016, 47 pts have been treated, 4 of whom had received 1 cycle of prior therapy and were in CR at the time of enrollment. The median age was 68 years (range, 60-81), and median CD22 expression was 97% (range, 72-100%). Of 43 pts evaluable for response, 42 responded (ORR=98%). Best response was CR in 36 pts (84%), CRp in 5 (12%) and CRi in 1 (2%). MRD negativity by 6-color multiparameter flow cytometry was achieved in 31 of 41 evaluable pts (76%) on day 21 and in 44 of 46 evaluable pts (96%) within 12 weeks of treatment. The median follow-up was 24 months (range, 1-55 months). 3 pts (6%) underwent allogeneic stem cell transplantation (ASCT) in first remission. Of the 46 responders, 6 pts (13%) have relapsed. 16 pts have died, 1 due to resistant disease, 4 after relapse, 1 after ASCT and 10 in CR/CRp. 21 pts remain on treatment (consolidation, n=3; POMP maintenance, n=19), and 5 pts have completed all therapy. The 3-year continuous CR and OS rates were 72% and 54%, respectively. Compared to a historical cohort of 79 older pts treated at our institution with hyper-CVAD ± rituximab, mini-hyper-CVD+InO resulted in significantly improved OS (3-year OS rate: 54% vs 31%; median OS not reached versus 16 months; P=0.007).

Summary/Conclusions: The combination of InO with mini-hyper-CVD is safe and effective in older pts with newly diagnosed ALL, resulting in a promising 3-year OS rate of 54%. These results appear superior to the outcomes of older pts treated with hyper-CVAD.

Acute myeloid leukemia - Biology 1

P171

RECURRENT MYB REARRANGEMENT IN BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM

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Background: Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare hematological malignancy that is derived from plasmacytoid dendritic cell precursors. BPDCN tends to occur in elderly people with frequent skin involvement and is associated with an aggressive clinical course and a poor prognosis. Although optimized diagnostics and therapies should improve patient outcomes, the pathobiological and genetic aspects of BPDCN remain unclear.

Aims: We planned this study to identify a critical genetic event in BPDCN, which could provide better understanding of BPDCN pathogenesis.

Methods: We enrolled fourteen patients (five children and nine adults) with BPDCN who were treated in our institutions. We primarily performed RNA sequencing-based comprehensive transcriptome analysis with their samples at the onset to detect gene fusions. These results were then used as the basis for genetic validation studies and functional analyses with an exogenous expression model.

Results: We identified a recurring gene rearrangement that involved the MYB proto-oncogene in all five pediatric patients (100%) and four of nine adult patients (44%) with BPDCN. The resulting fusion genes included MYB-ZFAT (four patients), MYB-PLEKHO1 (three patients), MYB-DCLS (one patient), and MYB-MIR3134 (one patient), none of which have been previously reported to our knowledge. The translocations corresponding to these fusions were not detected by the metaphase analysis except in one patient with t(1;15), who harbored MYB-PLEKHO1. These fusion genes were detectable at diagnosis and relapse but not at remission. Fluorescence in situ hybridization (FISH) analysis efficiently detected the breaking apart of MYB in formalin-fixed, paraffin-embedded sections. Consequent to the rearrangement, the negative regulatory domain of MYB was truncated, leading to constitutive MYB transcriptional activation, as described in other malignancies. Exogenous MYB-PLEKHO1 expression in HEK 293T cells led to the upregulation of several known downstream MYB targets. Gene set enrichment analysis also confirmed the activation of MYB target gene sets. The identified significantly upregulated genes included cell surface molecule-encoding genes such as NCAM1 (also termed CD56), CD68, S1PR1, and CXCR4, possibly providing targets for antibody-mediated anticancer therapies. We performed whole-exome sequencing of paired tumor–normal samples at diagnosis for four pediatric patients, which revealed a total of 91 (6–45 per patient) somatic mutations, a relatively large number compared with other pediatric cancers. However, no driver mutations were identified from the existing literature and database entries; only one nonsense mutation in KRT20 p.Cys1403Gly, was present on a driver gene, although this exact mutation had not been previously reported. Furthermore, we performed targeted sequencing covering genes associated with hematological malignancies in the remaining 10 patients. Consequently, children were not found to carry any identifiable driver mutations, whereas all adult patients harbored at least one point mutation in genes such as TET2, ASXL1, IKZF1, ZRSR2, NRAS, and EZH2, most of which were reported to be mutated in BPDCN and myeloid malignancies.

Summary/Conclusions: We identified a high frequency of MYB rearrangements that promoted the MYB transcriptional activity in BPDCN. MYB split FISH analysis can constitute a valuable diagnostic tool for detecting MYB rearrangements. We expect that our findings provide critical insights regarding BPDCN pathogenesis and contribute to molecular biology-oriented diagnostic techniques and molecular-targeted therapies for this intractable malignancy.
Background: The branched chain amino acids (BCAAs) valine, leucine, and isoleucine are essential AAs for the human body. The activity of BCAA metabolism high levels of the enzyme BCAA Transaminase 1 (BCAT1) have recently been associated with aggressiveness in several cancer entities. However, the mechanistic role of BCAT1 in this process remains uncertain.

Aims: To elucidate the mechanistic link between BCAT1 function and epigenetic deregulation in leukemia stem cells (LSCs) and consequences on clinical outcome.

Methods: High-resolution proteomics of LSCs, Knockdown and overexpression of BCAT1 in AML patient samples and AML cell lines, Gene set enrichment analysis, BCAA tracing experiments, Xenotransplantations, Metabolomics, DNA methylation arrays, correlative and mechanistic link to clinical data sets.

Results: We performed high-resolution proteomics analysis of human acute myeloid leukemia (AML) stem cell (LSC) and non-LSC populations, which have been functionally validated by xenotransplantation into NSG mice, and we found the BCAA pathway enriched and BCAT1 overexpressed in LSCs. We show that BCAT1, which transfers α-amino groups from BCAAs to α-ketoglutarate (αKG), is a component of the nuclear export signal (NES) at the C-terminus, indicating that the cytoplasmic localization is critical for the leukemic phenotype. The most frequent mutated AML cell line OCI-AML3 and create a therapeutic target to compromise LSC function in IDHwtTET2wtAML patients.

P174
THE LONG NON-CODING RNA HOXB-AS3 REGULATES RIBOSOMAL BIOGENESIS IN NPM1-MUTATED ACUTE MYELOID LEUKEMIA
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Background: Background: The prognostic significance of long non-coding RNA expression (lncRNAs) in older (≥60 years) patients (pts) with cytogenetically normal acute myeloid leukemia (CN-AML) was recently reported (Garzon et al., 2014). The IncRNA HOXB-AS3, which is embedded in the HOXB-locus, was found to be overexpressed among the IncRNAs that associated with mutated NPM1 (NPM1mut) in CN-AML.

Aims: Aims: Our aims were to evaluate the biologic significance of HOXB-AS3 expression in NPM1mutAML.

Methods: Methods: HOXB-AS3 expression profiling was performed by real-time PCR. Knock-down (KD) of HOXB-AS3 was performed in vitro and in vivo [in a pt-derived xenograft (PDX) model] with locked nucleic acid-modified gappers. Comparative proteomic analysis was conducted with a modified version of the RNA antisense purification (RAP) protocol (McHugh et al., 2015). Direct visualization of the HOXB-AS3 was performed using custom-designed Basecross (Advanced Cell Diagnostics, Newark, CA) according to the manufacturer’s instructions.

Results: Results: Of 6 AML cell lines that were tested, only OCI-AML3 cells, which harbor NPM1mut, showed detectable levels of HOXB-AS3 expression. Five- and 3-prime Rapid Amplification of cDNA Ends (RACE) assays in OCI-AML3 identified a previously annotated (NR_033201/NR_033203/ENST0000491264) and 1 novel variant of HOXB-AS3. NPM1mut pt samples exhibited higher expression of HOXB-AS3 compared to those with wild-type (WT) NPM1 (P<0.001) and healthy donors (P<0.001). In vitro KD of HOXB-AS3 led to decreased proliferation of OCI-AML3 cells, as measured by BrdU-based cell cycle analysis (S-phase average% in control vs KD: 24% vs 16%, P<0.02). HOXB-AS3 KD also led to a reduction in the number of formed colonies by OCI-AML3 cells in colony-forming assays (P<0.002). HOXB-AS3 KD in NPM1mut pt blasts (n=5) led to a decrease in the number of formed colonies (P<0.001). To evaluate the effect of HOXB-AS3 KD in vivo we generated a murine PDX model by engrafting NSG mice with blasts of a NPM1mut pt. Treatment of the engrafted mice with nanoparticle-formulated anti-HOXB-AS3 gappers led to significant prolongation of survival compared to treatment with non-targeting control gappers in 2 independent experiments (P<0.01 and P<0.005). Mass spectrometry and comparative proteomic analysis of HOXB-AS3- and U1-specific RNA-protein complexes identified HOXB1 and NPM1 as candidate HOXB-AS3-binding proteins. RNA-immunoprecipitation experiments validated the interaction of HOXB-AS3 with EBPI (20-fold increase of HOXB-AS3 abundance in EBPI-precipitate compared to normal IgG control, P<0.001). Direct visualization of HOXB-AS3 showed co-localization of the IncRNA and WT NPM1 in the nucleoli. HOXB-AS3 was previously shown to interact with NPM1 and to regulate ribosomal biogenesis and growth of AML cells (Nguyen et al., 2016). We hypothesized that HOXB-AS3 could affect the EBPI-NPM1 interaction and impact on the ribosomal biogenesis process. In consistency with this hypothesis, HOXB-AS3 KD led to a decrease in the transcription of rRNA species in OCI-AML3 cells (P<0.001) and in vitro-treated blasts of 2 NPM1mut pts (P<0.001). HOXB-AS3 KD also led to a reduction of protein synthesis in the AML cells, as measured by incorporation of fluorochrome-tagged tracers in newly translated polypeptides.
Summary/Conclusions: Conclusions: HOXB-AS3 is strongly associated with NPM1 mutations in AML. HOXB-AS2 interacts with ESP1 and NPM1 and regulates ribosomal biogenesis in the leukemic blasts. From a therapeutic standpoint, HOXB-AS3 constitutes a promising target, as in vivo anti-HOXB-AS3 treatment prolonged survival in a murine PDX model.

P175

A DUAL BH3-MIMETIC APPROACH TARGETING BOTH BCL-2 AND MCL1 IS HIGHLY EFFICACIOUS AND WELL-TOLERATED IN ACUTE MYELOID LEUKEMIA

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Background: Identification of a chemotheraphy-free option for acute myeloid leukemia (AML) represents a highly desired and important research objective. Perturbation of cell survival is an essential hallmark of cancer now amenable to precision targeting by small molecule BH3-mimetics able to inhibit pro-survival BCL-2 (e.g. S55746) and MCL1 (e.g. S63845) proteins. BCL-2 (e.g. S55746) and MCL1 (e.g. S63845) inhibitors have shown promising activity in pre-clinical AML models. Here, we report on the efficacy of a dual BH3-mimetic approach in AML cell lines and preclinical models of AML.

Methods: AML cell lines were obtained from ATCC or DMSZ. S55746 (BCL-2 inhibitor) and S63845 (MCL1 inhibitor with 6-fold higher affinity to human than mouse Mcl1) were obtained from Servier and A1155463 (BCL-XL inhibitor) from Guillaume Lessene (WEHI). Primary AML cells were obtained from patients providing informed consent. For in vivo experiments, NSG; NOD.Cg-Prkdcscid Il2rgtm1WjlSzJ (NSG) or NOD/Rag-1-/-Il2rgtm1Wjl (NRRGS) mice were used.

Results: S55746 and S63845 showed strong synergy (Loewe score >5) in 13 AML cell lines tested, suggesting this dual BH3-mimetic targeting approach was highly efficacious (Figure 1A). S55746 and S63845 lowered the LC50 in primary AML samples by 10-100-fold in the majority of cases tested, confirming remarkable anti-leukemic activity across a spectrum of AML cases with diverse cytogenetic and molecular pathologies (Figure 1B).

A smaller fraction of AML samples were also sensitised to combined A1155463 and S63845 therapy. Bluminiscent imaging showed rapid and sustained clearance of xenografted MV4;11 AML (FLT3-ITD mutant and MLL re-arranged) cells, translating into significant prolongation of survival (Figure 1C) from combined S55746+S63845, but not from treatment with either BH3-mimetic alone. Similar in vivo efficacy was observed with xenografted OCI-AML3 cells harboring mutant NPM1 and MLLT35A. Patient-derived xenografts showed rapid reduction of established AML in the bone marrow one week of treatment with S55746 and S63845 (Figure 1D). Safety and tolerability of this approach was confirmed using normal CD34* stem and progenitor cells in short-term cell culture (48h) and long-term (2-3 weeks) clonogenic assays and from histological and biochemical examination of mice receiving treated for up to 8 weeks at doses shown to be highly efficacious against AML.

Summary/Conclusions: Dual BH3-mimetic targeting of BCL-2 and MCL1 induces rapid and synergistic cytoreduction of human AML cell line and primary AML samples in vitro and in vivo and across a diverse range of AML genotypes. These preclinical results support the translational investigation of dual BH3-mimetic targeting of BCL-2 and MCL1 in the clinic for the treatment of patients with AML.

P176

THE PMLC62A/C65A KNOCK-IN MOUSE MODEL PROVIDES EVIDENCE FOR THE ROLE OF NUCLEAR BODY DISRUPTION IN THE PATHOGENESIS OF ACUTE PROMYELOCYTIC LEUKEMIA

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Background: Acute promyelocytic leukemia (APL) is driven by the oncogene PML-RARA which is generated by fusion of the promyelocytic leukemia (PML) and retinoic acid receptor alpha (RARA) genes, and which strongly interferes with downstream signalling and the architecture of multiprotein structures known as PML nuclear bodies (NBs). NB disruption is a diagnostic hallmark of APL; however, the importance of this phenomenon has only been studied in vitro.

Aims: The aim of this study was to decipher the impact of Pml NB disruption in APL pathogenesis.

Methods: We engineered a knock-in mouse model with NB disruption achieved through mutation of key zinc-binding cysteine residues (C62A/C65A) in the Pml ring domain.

Results: While no leukemias or tumors developed in PmlC62A/C65A mice, the forced dimerization of RARα - mediated artificially by linking RARα to the dimerisation domain of the Nfsb p50 subunit - in cooperation with NB disruption was associated with doubling in the rate of leukemia (p<0.0001), with a reduced latency period (p=0.008). Moreover, response to targeted therapy with ATRA was significantly improved in PmlC62A/C65A mice transplanted with Pml WT-RARα leukemic blasts, but not with PmlC62A/C65A-p50-RARα, revealing the essential role of NBs for an effective response to differentiating drug. While formation of the PML-RARA fusion is considered a initiating event in APL pathogenesis, it is insufficient for the full leukemic phenotype. Moreover, whole exome sequencing analyses have consistently identified presence of cooperating mutations. Since Pml and Pml NBs have established roles in DNA repair and in the maintenance of genomic stability, we speculated that loss of NB integrity could affect these functions. Here, whole exome sequencing revealed a trend of higher genomic instability in PmlC62A/C65A mice as compared to PmlWT. p50-RARα leukemic blasts, but not with PmlC62A/C65A-p50-RARα, revealing the essential role of NBs for an effective response to differentiating drug.

Summary/Conclusions: Our study highlights the importance of re-formation of NBs for an efficient response to targeted therapy, the significant contribution...
of Pml-NB to the effectiveness of DNA damage repair processes, and the manner in which their disruption mediated by the PML-RARα oncoprotein can assist APL pathogenesis.

P177
DEICHERING THE ONCOCENIC NETWORK OF PRC2 LOSS GUIDED LEUKEMOGENESIS
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Methods: To model the complex interplay of mutational networks we performed CRISPR-Cas9 screenings with oncogene/tumor suppressor pools in vitro and in vivo. Cellular resources generated were subjected to mutational and molecular profiling.

Results: To this end, a 96-well based CRISPR-Cas9 immortalization assay allowing fast and quantifiable genetic cooperation screenings was established. Four out of six CRISPR-Cas9 pools tested—composed of five genes each and representing 148 mutation combinations—reproductively transformed LS K cells with distinct clonal output. Transformation of in vitro immortalized clones yielded robust engraftment and multilineage contributions in mice but no overt leukemia was detected, indicating that induced mutations select for a preleukemic state in vitro. We thus tested every oncogene/tumor suppressor pool from the in vitro setting in a murine bone marrow transplantation model with freshly transduced LS K cells which resulted in robust induction of leukemia. Analysing the mutational spectrum of derived clones we were able to raise a list of potential partners cooperating with Ezh2 loss, which highlighted NFI (Ras-signalling), loss of Dnm3α, and loss of Runx1 as cooperating partners, whereas loss of cohesin complex subunits (Smc3, Stag2) seems to be dispensable during the induction of Ezh2-loss guided leukemogenesis. To define oncogenic dependencies in myeloid malignancies with PRC2-loss we analysed gene expression spectra of the generated samples. While in vitro transduced clones presented with distinct expression signatures clearly separating from controls a partially overlapping expression signature could be established. Through identification of these cooperating mutations and the resulting gene expression signature, which will be validated in a CRISPR-Cas9 knock-out screening we aim to identify novel therapeutic targets in AML.

Summary/Conclusions: Our study highlights the power of the CRISPR-Cas9 system to probe oncogenic interaction. Mutational CRISPR screenings in vitro, and a newly established in vitro CRISPR-Cas9 immortalization assay for high throughput screening of sgRNA pools, delivered potential cooperating partners of Ezh2 loss in AML, and provides rich cellular resources to identify molecular mechanisms of oncogenic synergies and dependencies.

P178
Abstract withdrawn.

P179
ACUTE MYELOID LEUKEMIA EVOLUTION CAN BE RECONSTRUCTED BY ANALYSIS OF NON-LEUKEMIC CELLULAR SUBCOMPARTMENTS AND MULTILINEAGE ENGRAFTED MICE
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Background: Hematopoietic Stem Cells (HSC) isolated from patients with Acute Myeloid Leukemia (AML) have been shown to carry leukemia-specific mutations leading to the concept of pre-leukemic HSC. In order to understand the evolution from multi-potent pre-leukemic HSC to fully transformed AML, an accurate molecular comparison of patient matched HSC and leukemic cells is essential. Recently we have shown that functionally normal HSC can be separated from a subgroup of AML patients using the surface marker combination CD34+CD38+ and high ALDH enzyme activity (CD34+CD38+ALDH+).

Aims: In this study we aim to understand the leukemic evolution from pre-leukemic HSC to fully transformed AML.

Methods: Whole exome sequencing (WES) of 12 diagnostic AML samples with the matched germ-line controls (T cells or buccal swab) was performed. Leukemia-specific mutations were identified according to specific criteria (Allele frequency >0.20, Sanger evidence >0.5, coverage >10 reads, support >2 reads, and GMAF <0.05) and validated. Identified AML-specific mutations were tracked in different cellular compartments (T- and B-cells) as well as in single HSC colonies derived from diagnostic AML samples. To test the functional properties of pre-leukemic HSC in vivo, we transduced bulk AML in NOD/SCID-IL2Rγnull (NSG) mice and analyzed human subpopulations (myeloid and lymphoid) of multilineage engrafted animals for the presence of leukemia-specific mutations.

Results: WES identified 64 AML-specific mutations. Most cases (8 out of 12) showed 4-6 AML specific mutations per sample (1-18 mutations/AML) including point mutations in genes that are recurrently mutated in AML (e.g. DNMT1, NRAS and KIT). Tracking of AML-specific mutations in non-leukemic T- and B-cells showed that some AML mutations like DNMT3A, IDH1, IDH2, EZH2 and ZNF536 were already detectable in T- and B-cells indicating their pre-leukemic status. Furthermore, analysis of multi-lineage engrafted xenografts detected leukemia-specific mutations in human myeloid and lymphoid sub-compartments suggesting that these animals were engrafted from functionally normal pre-leukemic HSC. To reconstruct the sequence of pre-leukemic mutations single-cell HSC were seeded and the resulting colonies analyzed for the presence of the respective leukemia specific mutations. Based on the different mutational data, combined with the cellular context in which these were detectable the leukemic evolution of most patients could be reconstructed. In one patient we detected a DNMT3A mutation in myeloid and lymphoid cells, whereas NPM1 and FLT3-ITD mutations were only detectable in leukemic cells proving the pre-leukemic status of DNMT3A in this case. In another patient we found DNMT3A IDH2 in T- and B-cells whereas TP53 and FLT3-ITD mutation were only detectable in leukemic cells. By analyzing colonies from single cell HSC we were able to detect complex pre-leukemic hierarchies with one example in which a ZNF536 mutation could be identified as initiating event that hasn’t been described in leukemia yet.

Summary/Conclusions: We can identify leukemia specific mutations including mutations in genes that haven’t been described in AML yet. Tracking of these mutations in various non-leukemic cellular compartments including HSC and multi-lineage engrafted mice allows reconstruction of the individual leukemic evolution. A better understanding of these processes may pave the way for new treatment strategies with the aim to target the relevant leukemic events.

P180
THE ESSENTIAL ROLE OF THE ENHANCERS OF POLYCOMB EPC1 AND EPC2 IN MLL-AF9 ACUTE MYELOID LEUKAEMIA IS A ‘COMPLEX’ STORY
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Background: The Enhancers of Polycomb (EPC) proteins EPC1 and EPC2 are critical in the survival of and leukemogenesis driven by MLL-AF9, a bivalent oncogene that drives acute myeloid leukemia (AML). Most importantly, loss of EPC1 or EPC2 in MLL leukemia stem cells, but not normal hematopoietic stem cells and progenitor cells, leads to the induction of cellular apoptosis. To date little is known about the functional contribution of EPC1 and EPC2 in AML. EPC1 is an essential component of the histone acetyltransferase complex. Additionally, EPC1 has been found in complexes with the Enhancer of zeste homolog 2 (EZH2), a catalytic core subunit of the histone methyltransferase Polycomb repressive complex 2 (PRC2). NuA4 and PRC2 are two major chromatin modifying complexes encompassing opposing epigenetic activities and both are known to be deregulated in AML. A systems biology approach to understand the essential contribution of the homologous chromatin regulatory proteins EPC1 and EPC2 in AML is needed.

Methods: Mass spectrometry (MS) analysis was performed on immunoprecipitated protein using EPC1 antibody from human THP1 MLL-AF9 AML cell line. Chromatin immunoprecipitation (ChIP) was performed using HighCell ChIP Kit and iPure kit v2 (Diagenode) followed by NextSeq500 Illuma sequencing in THP1 cells. ChIP enriched regions were identified using SICER peak calling and ChIPpeakAnno. Lenti-derivatives were prepared and THP1 cells were infected with viral particles containing pLKO.1 puro lentiviral vector expressing short hairpin RNAs. RNA was extracted 72 hr following lentiviral transductions and whole transcriptome sequencing was performed. DESeq2 was used for differential expression analysis.

Results: MS analysis identified the core NuA4 complex components (TIP60, ING3, RUVBL1, RUVBL2, EP400 and DNAI1) and also revealed additional interacting partners such as HAT1 and HDAC2. A better understanding of these processes may pave the way for new treatment strategies with the aim to target the relevant leukemic events.
histone methylation and acetylation profiles following lentiviral shRNA knockdown (KD) of EPC1 or EPC2 in THP1 cells. Interestingly, we find significant changes in histone H3K27 trimethylation levels as well as changes in the levels of histone H3 and H4 acetylation following KD of either EPC1 or EPC2 expression. Notably, the identified regions demonstrating changes in histone H3K27me3 levels are enriched for PR2C target genes. RNA sequencing followed by gene-set enrichment analysis indicated significant transcriptional changes in PR2C regulated genes following lentiviral shRNA knockdown of EPC1 or EPC2. Meta-analysis of this PRC signature identified a sub-group of genes that are directly regulated by the EPC complex which include the monocytic differentiation inducer MAFB, the H2A ubiquitin ligase TRIM37 and the pro-apoptotic tumor suppressor CMTM3.

Summary/Conclusions: Our data suggests that EPC1 and EPC2 are required for the recruitment of certain chromatin proteins to form EPC-associated complexes which are essential for the maintenance of an AML epigenetic signature and an aberrant transcriptional profile that supports leukemia stem cell survival. We have identified and characterized the EPC complex components in human AML. Additionally, we have refined a sub-group of PRC target genes that are regulated by the EPC complex which represent potential novel therapeutic targets in human AML. Overall we present a comprehensive analysis of the aberrant epigenomic landscape of THP1-MLL-AF9 AML cells in relation to EPC1 and EPC2 and provide new insight into their deregulated role in AML.

Background: The bone marrow (BM) microenvironment is known to protect AML cells from drug therapy. We showed earlier that conditioned medium (CM) from the BM stromal cell line HS-5 increased cell viability and led to resistance to specific drug classes. Aims: Here, we investigate the mechanisms mediating the BM stromal cell induced resistance to venetoclax and its reversal by ruxolitinib.

Methods: Phospho-flow analysis was done by stimulating AML patient cells with GM-CSF, G-CSF, IL-6, IL-8 or MIP-3α (10 ng/ml) for 20 min, after which the cells were stained with Alexa 647-anti-phospho-Stat5 (pY694), PE188 CF594-anti-phospho-Stat3 (pY705), BV421-anti-phospho-Akt (pS473) and PE-anti-phospho-Erk1/2 (pT202/pY204). For co-culture and transwell assays AML cells were added directly to MNCs from AML patients or separated by a 0.4 μm pore membrane. Vehicle (DMSO), ruxolitinib (300 nM), venetoclax (100 nM) or their combination were incubated for 48h and AML cells labeled with PE Annexin V, 7AAD, PE-Cy7-CD34, BV605-CD45. In vivo drug efficacy was tested on NSG mice inoculated i.v. with MOLM-13αAML cells. Mice were divided into control, venetoclax (25 mg/kg, i.p.), ruxolitinib (50 mg/kg BID, p.o.), combination groups (all n=6) and treated for 3 weeks, 5 days a week with 2 days off.

Results: To identify the factors contributing to BM mediated drug resistance of AML cells, we analyzed the effect of IL-6, IL-8, MIP-3α, GM-CSF and G-CSF, cytokines enriched in the HS-5 CM, on proliferation of MNCs collected from AML patients. GM-CSF and G-CSF alone conferred resistance to venetoclax similar to CM that we showed earlier to reduce sensitivity to BCL2 inhibitors. To identify the impact of stroma-derived factors on cellular signaling we stimulated AML patient cells with CM and analyzed the phosphorylation of STAT3, STAT5, ERK and AKT. Compared to control conditions, CM rapidly induced phosphorylation of STAT5 in primary AML cells. When the effect of individual cytokines was tested, we noted that GM-CSF and G-CSF alone mimicked the effect of CM on cellular signaling. Gene expression data showed the receptor for GM-CSF (CSFR2A) was more highly expressed in AML patient cells compared to healthy controls. Taken together, these results show that cytokines such as GM-CSF from BM stromal cells increase JAK/STAT signaling, which may lead to enhanced survival of AML cells. To determine whether the protective effect of stroma on BCL2 inhibition was dependent on cell-to-cell interactions we cultured AML patient cells either in direct contact with MNCs or separated from stroma with a 0.4 μm pore membrane. 48h treatment with 100 nM venetoclax did not result in significant reduction of CD34+ AML cells regardless of whether AML cells were directly cultured with stroma or separated by a membrane, further indicating that stroma-derived soluble factors are sufficient to reduce sensitivity to venetoclax. Since the most abundant cytokines secreted by HS-5 cells, GM-CSF and G-CSF led to increased phosphorylation of STAT5 in primary AML cells, when the effect of individual cytokines was tested, we noted that GM-CSF and G-CSF alone could mimic the effect of CM on cellular signaling. Gene expression data showed the receptor for GM-CSF (CSFR2A) was more highly expressed in AML patient cells compared to healthy controls. Taken together, these results show that cytokines such as GM-CSF from BM stromal cells increase JAK/STAT signaling, which may lead to enhanced survival of AML cells. To determine whether the protective effect of stroma on BCL2 inhibition was dependent on cell-to-cell interactions we cultured AML patient cells either in direct contact with MNCs or separated from stroma with a 0.4 μm pore membrane. 48h treatment with 100 nM venetoclax did not result in significant reduction of CD34+ AML cells regardless of whether AML cells were directly cultured with stroma or separated by a membrane, further indicating that stroma-derived soluble factors are sufficient to reduce sensitivity to venetoclax. Since the most abundant cytokines secreted by HS-5 cells, GM-CSF and G-CSF led to increased phosphorylation of STAT5 in primary AML cells, when the effect of individual cytokines was tested, we noted that GM-CSF and G-CSF alone could mimic the effect of CM on cellular signaling. Gene expression data showed the receptor for GM-CSF (CSFR2A) was more highly expressed in AML patient cells compared to healthy controls. Taken together, these results show that cytokines such as GM-CSF from BM stromal cells increase JAK/STAT signaling, which may lead to enhanced survival of AML cells. To determine whether the protective effect of stroma on BCL2 inhibition was dependent on cell-to-cell interactions we cultured AML patient cells either in direct contact with MNCs or separated from stroma with a 0.4 μm pore membrane. 48h treatment with 100 nM venetoclax did not result in significant reduction of CD34+ AML cells regardless of whether AML cells were directly cultured with stroma or separated by a membrane, further indicating that stroma-derived soluble factors are sufficient to reduce sensitivity to venetoclax. Since the most abundant cytokines secreted by HS-5 cells, GM-CSF and G-CSF led to increased phosphorylation of STAT5 in primary AML cells, a downstream effector of JAKs, we tested a combination of venetoclax and JAK1/2 inhibitor ruxolitinib. We found that ruxolitinib potentiated sensitivity to venetoclax when tested with AML patient cells in HS-5 CM and in co-culture and transwell assays. Significantly, the combination was more effective at reducing tumor burden in a xenograft mouse model of AML than either drug alone.

Summary/Conclusions: In conclusion, our data demonstrate that BM secreted soluble factors drive cytoprotection against BCL2 antagonist venetoclax that can be overcome by combined blockade of JAK/STAT and BCL2 pathways with ruxolitinib and venetoclax in vivo co-culture models and in vivo in an AML mouse model.
adult AML cases. Despite having poor outcomes, CK-AML is the least understood at the molecular level, except for the finding that about two-thirds of cases carry TP53 alterations. In particular, because cytogenetic alterations appear to be distinct among different patients, it is unclear whether they are cause of leukemogenesis, or merely reflect a state of genomic instability.

**Aims:** We have hypothesized that cytogenetic aberrations in CK-AML create genetic lesions that recur across patients, potentially de novo cancer genes that contribute to leukemogenesis in individual patients.

**Methods:** We performed a transcriptome analysis using Illumina paired-end (101bp2) RNA sequencing of 65 CK-AML cells to identify gene fusions using multiple independent algorithms (as paired reads that flank, or single-reads that stop across junctions) that recur across patients. We identified gene fusions in part independently validated by array-based genomic profiling and/ or long range PCR as shown by use of long-read Oxford Nanopore sequencing technology.

**Results:** We identified 54 gene fusion events in 30 of the 65 cases (46%) with up to four fusions per case. All fusions are supported by 10-50+ junction-spanning reads. Many of the fusions are independent of each other and are in strong agreement with genomic DNA breakpoints from array-based genomic profiling and/ or long range PCR, respectively. Approximately 35% of the fusions were in-frame, encoding chimeric proteins. The remaining encoded either C-terminally truncated 5' fusion partners, or else N-terminally truncated (or rarely full-length) 3' fusion partners. Those fusions involved in the 5' partner contributed only to the 5'UTR. In many instances, the fusions are predicted to lead to the overexpression or chimeric activation of known or putative novel cancer genes. Of the 54 fusions, only three (RUNX1-MECOM, MEE1-ETV6, and ETV6-MEE1) were previously reported in AML. The most frequently affected genes were RUNX1 (n=5), KMT2A, and MECOM (n=3 each). Based on the affected gene, these gene fusions were categorized into six functional fusion clusters. Many of the fusions contained at least one known AML gene (n=16; e.g. RUNX1, MECOM, DEK, ETV6, KMT2A) together with a novel fusion partner, clearly suggesting pathogenic relevance. Other fusions were predicted to disrupt known tumor suppressors (n=4; e.g. TP53, TERT) or to activate known oncogenes (n=5; e.g. MB2). Others encoded chimeric proteins of unclear pathogenic relevance, but that could nonetheless encode novel epitopes created by the fusion junction (n=26).

**Summary/Conclusions:** Detailed molecular characterization of CK-AML revealed a high incidence of novel gene fusions in about 50% of cases. The affected genes suggest a more general role in leukemogenesis by reflecting a state of genomic instability. Furthermore, identifying gene fusions in each individual patient might lead to more effective, personalized treatments that target the gene fusion itself, enable immunologic therapies against the fusion junction epitopes, and provide private patient-specific biomarkers to track leukemic burden for the monitoring of disease remission and relapse.

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**P183**

**H3K27ME3 LEVEL ON THE HIST1 CLUSTER: A POWERFUL EPIGENOMIC BIOMARKER THAT STRATIFIES TWO GROUPS OF NPM1-MUTATED AML DIFFERING IN THEIR OUTCOME AND EXPRESSION PROFILE**

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**Background:** NPM1 mutation (NPM1mut) is the most frequent genetic alteration found in cytogenetically normal acute myeloid leukemia (CN-AML). Patients harboring NPM1mut without FLT3 internal tandem duplication (FLT3-ITD) are considered to have favorable outcome. Yet, some of them relapse and become resistant to chemotherapy. Little is known about biological processes underlying treatment failure. Our group previously described a new AML gene (n=16; e.g. RUNX1, MECOM, DEK, ETV6, KMT2A) together with a novel fusion partner, clearly suggesting pathogenic relevance. Other fusions were predicted to disrupt known tumor suppressors (n=4; e.g. TP53, TERT) or to activate known oncogenes (n=5; e.g. MB2). Others encoded chimeric proteins of unclear pathogenic relevance, but that could nonetheless encode novel epitopes created by the fusion junction (n=26).

**Summary/Conclusions:** Detailed molecular characterization of CK-AML revealed a high incidence of novel gene fusions in about 50% of cases. The affected genes suggest a more general role in leukemogenesis by reflecting a state of genomic instability. Furthermore, identifying gene fusions in each individual patient might lead to more effective, personalized treatments that target the gene fusion itself, enable immunologic therapies against the fusion junction epitopes, and provide private patient-specific biomarkers to track leukemic burden for the monitoring of disease remission and relapse.

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**P184**

**FUNCTIONAL ASSESSMENT OF NOVEL DIAGNOSTIC FLT3 MUTATIONS AND INHIBITION BY KINASE INHIBITORS**

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**Background:** Somatic mutations in FLT3 are among the most common events in AML, with FLT3ITD mutations in the juxtamembrane domain (JMD) as well as DB35 missense mutations in the kinase domain (KD) the predominant events. Sequencing of FLT3 in a cohort of 788 children with de novo AML treatment on contemporary Children’s Oncology Group protocols demonstrated that, in addition to the previously described FLT3 mutations (ITD and DB35), numerous other variants, including several novel variants, were present in 8% of patients at diagnosis, leading to a cumulative FLT3 mutation prevalence of 27% in children and young adults. These variants mostly occurred in the JMD and KD, and sequencing the number of patients who might be amenable to FLT3 inhibitor therapy. Aims: We evaluated the oncogenic capability of each of these mutations by assessing their ability to result in aberrant FLT3 and STAT5 phosphorylation, as well as response to the tyrosine kinase inhibitors crenolanib and quizartinib.

**Methods:** Point mutations were introduced into HSC293 cells using retroviral transduction. Following transduction, phosphorylation status of FLT3 (pFLT3) and downstream STAT5 (pSTAT5) were evaluated by immunoblotting. Phosphorylation status was quantified by chemiluminescence analysis and the quan-
tility of protein expression was normalized to actin. That ratio of phosphorylated protein to total protein for FLT3 and STAT5 was determined and normalized to that observed in the D835Y mutation as a positive control. A value of >10% pFLT3 was considered positive. All mutations that resulted in FLT3 phosphorylation were subsequently evaluated for inhibition by crenolanib and quizartinib following 60-minute exposure to the compounds.

Results: A total of 24 non-ITD and non-ABL AML mutations were evaluated for autonomous FLT3 and STAT5 phosphorylation. Eleven mutations resulted in pFLT3 and pSTAT5, including 4 mutations with >50% pFLT3. All mutations that demonstrated aberrant pFLT3 also had aberrant pSTAT5, however a direct correlation of pFLT3 and pSTAT5 was not always observed. Overall, 67% (n=86 patients) of all non-ITD mutations evaluated resulted in autonomous FLT3 activation. Excluding D835 mutations, 64% (n=39) of patients harbored an activating mutation. Many of the mutations that were not found to be activating had the lowest prevalence, often present in only one patient. Evaluation of inhibition of pFLT3 by crenolanib demonstrated that in every case of aberrant activation, crenolanib resulted in potent inhibition of phosphorylation of FLT3 and STAT5 with an IC50 range of 1.3-13.9 nM and 0.6-6.5 nM respectively. Many of the mutations tested were exquisitely sensitive to crenolanib, with 9 of 10 mutations tested demonstrating an IC50 of pFLT3 inhibition ≤5.6 nM. Inhibition of downstream kinases is necessary for optimal efficacy of any FLT3 inhibitor and phosphorylation of STAT5 was potently inhibited by crenolanib in all cases. Quizartinib inhibited pFLT3 and pSTAT5 with an IC50 range of 1.8-151.7 nM and 1-33.9 nM respectively, demonstrating less effective inhibition specifically at mutations including D835Y, D839E, N670K, M684I.

Summary/Conclusions: We have previously presented that FLT3 mutations, including novel mutations in addition to the FLT3/ITD and D835, are prevalent in children and young adults with AML. Here we demonstrate that many of the non-ITD/D835 mutations also result in aberrant FLT3 phosphorylation and are amenable to inhibition by FLT3 inhibitors. Crenolanib resulted in potent inhibition of FLT3 and downstream STAT5 in all mutations tested. This data supports expanding the cohort of pediatric patients with activating FLT3 mutations who may benefit from FLT3 inhibitor therapy beyond those with FLT3/ITD.

P185
Abstract withdrawn.

P186
THE BCL-2 INHIBITOR VENETOCLAX INHIBITS NRF2 ANTIOXIDANT PATHWAY ACTIVATION INDUCED BY HYPMETHYLATING AGENTS IN ACUTE MYELOID LEUKAEMIA
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Background: The selective Bcl-2 inhibitor Venetoclax (ABT-199) has shown potent antileukemic activity against Acute Myeloid Leukemia (AML) in preclinical and early clinical studies and impressive results have been achieved using the combination of hypomethylating agents (HMA) with venetoclax suggesting synergy between these agents.

Induction of Reactive Oxygen Species (ROS) is important for the cytotoxicity of various AML therapies including HMA. Induction of ROS by various cytotoxic therapies concurrently activates the NRF2 antioxidant response pathway which in turn results in induction of antioxidant enzymes that neutralize ROS. Upon ROS induction, the transcription factor Nrf2 is released from its adaptor protein Keap 1 in the cytoplasm whereby Nrf2 enters the nucleus and binds to antioxidant response element sequences in the promoters of various genes. Nrf2 pathway activation has been shown to mediate chemoresistance in various cancers including AML. Low ROS levels have been shown to be a hallmark of leukemia stem cells and are critical to their self renewal capacity. In this study, we examined whether Nrf2 inhibition is an additional mechanism responsible for the marked antileukemic activity in AML seen with the combination of HMAs and venetoclax.

Aims: To determine the effect of venetoclax on ROS levels after HMA exposure in AML cells and to examine the effect of Bcl-2 inhibition on NRF2 antioxidant pathway activation in response to HMA.

Methods: The effect of combination venetoclax and HMA on ROS levels and apoptosis was measured by flow cytometry. Effect of venetoclax and HMA on Nrf2 nuclear translocation was analyzed by immunostaining after cellular fractionation. Effect of venetoclax treatment on the association of Bcl2 with Nrf2 (Keap 1 complex was assessed by Western blot analysis, immunoprecipitation and in vitro assay for ubiquitination.

Results: Our results demonstrated that combination of HMA with venetoclax augmented cellular and mitochondrial ROS induction and apoptosis compared to treatment HMA alone. Treatment of AML cell lines as well as primary AML cells with venetoclax and decitabine resulted in increased nuclear translocalization of Nrf2 (Figure 1) and induction of downstream antioxidant enzymes including HO-1 and NQO1. Immunofluorescence studies confirmed the inhibition of nuclear translocation of Nrf2 by venetoclax. Immunoprecipitation studies indicated that Bcl-2, Keap 1 and Nrf2 associate in a protein complex in the cytoplasm and that treatment with venetoclax leads to dissociation of Bcl-2 from the Nrf2/Keap 1 complex and targets Nrf2 to ubiquitination and proteasomal degradation.

Figure 1.

Summary/Conclusions: In conclusion, inhibition of Nrf2 pathway may explain the marked potentiation of HMA activity by venetoclax that is observed in clinical trials. We show that ROS induction at least partially mediates the cytotoxicity of HMA and ROS induction after HMA treatment is augmented by venetoclax. We demonstrate for the first time that venetoclax is a potent inhibitor of Nrf2 activation via disruption of the association between Nrf2, Keap 1 and Bcl-2.

P187
UNRAVELING EPigenomic REGULATION IN THE EVOLUTION OF RELAPSING PEDIATRIC AML
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Background: In comparison with pediatric acute lymphoblastic leukemia, pediatric acute myeloid leukemia (AML) is characterized by a high relapse rate (~30%), and lower overall survival rates of 60-70%. It is therefore crucial to increase our insights in pathophysiological mechanisms underlying AML relapse, including chemotherapy resistance, clonal evolution, and clonal selection. There is increasing evidence that epigenetic deregulation is involved in the initiation and progression of cancers, including adult AML. Epigenetic regulation involves the activity of non-coding regulatory DNA elements such as enhancers, which interact with promoters to fine-tune gene expression. Importantly, epigenetic signatures at enhancers are highly cell state specific. Since little is known concerning the epigenetic landscape of pediatric AML, it is crucial to gain more insights into the epigenome of relapsed and non-relapsed AML children.

Aims: To identify differential epigenomic regulatory pathways involved in AML relapse by exploring the epigenome of relapsed (RP) and non-relapsed pediatric AML patients (NRPs).

Methods: The epigenome of 20 AML patients, harboring known molecular aberrations (including MLL-rearrangement, CBF-related and Fli3-ITD), was analyzed by identifying active regulatory pathways. Acetylation of lysine 27 on the tail of histone H3 (H3K27ac) marks active regulatory DNA elements and was therefore used to identify active promoters and enhancers using Chromatin-Immunoprecipitation-sequencing (ChIP-seq) experiments. Additionally, single-cell RNA-seq data were generated for selected AML patients to analyze clonal heterogeneity.

Results: All genomic regions that were significantly enriched by H3K27ac were analyzed, resulting in ~30.000 active promoters and enhancers per sample. Genome-wide Pearson correlation of all enriched regions showed subcustering of patients based on molecular aberration. Interestingly, epigenomic analysis showed that the initial diagnosis (Dx) and the patient’s relapse (Rel) sample were highly correlated. Also, single-cell RNA-seq analysis identified two highly identical homogeneous populations at Dx and Rel. Following the fact that no major differences were observed between AML cell lines at diagnosis and relapse, NRPs were analyzed. Here striking differences in H3K27ac enrichment were observed in MLL-rearranged patients between NRPs and RPs. Enhancers and promoters were differentially enriched at diagnosis, of which Spo11, a kinase involved in proliferation and survival, was significantly more enriched in RPs, while the promoter of transcription factor ELF1 and nearby located enhancers were active in NRPs only.
MECHANISTICALLY INFORMED COMBINATIONS OF SY-1425, A POTENT AND SELECTIVE RARA AGONIST, WITH HYPOMETHYLATING OR ANTI-CD38 TARGETED AGENTS IN AML AND MDS

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Background: The complex pathogenesis of cancer often necessitates combination therapies to optimize patient benefit. Thus, we investigated preclinical combinations of SY-1425 (tamborotene) and other agents to build on the monotherapy strategy with SY-1425 in biomarker selected AML and MDS patients (Phase 2 study, NCT02807558). Based on the RARα mediated myeloid gene activation of SY-1425, epigenetic priming with hypomethylating agents (HMAs) and CD38 induction were explored.

Aims: We sought to investigate mechanically informed combinations of SY-1425 and other agents (HMAs and novel agents in AML) and that strong upregulation of the maturation marker CD38 in AML cells by SY-1425 mediated reprogramming by relieving aberrant methylation of RARα target genes and that strong upregulation of the maturation marker CD38 in AML cells by SY-1425 could induce sensitivity to the anti-CD38 therapeutic antibody daratumumab (DARA).

Methods: HMA synergy was tested in vitro in AML cell lines over a range of concentrations for SY-1425 and azacitidine. In vivo studies used a disseminated patient derived xenograft (PDX) model of AML expressing high levels of RARA. SY-1425 induction of CD38 was assessed by H3K27ac/ChiP-seq, RARA ChiP-seq and flow cytometry. Antibody dependent cell-mediated cytotoxicity (ADCC) was tested in an ex vivo co-culture model of human NK cells and AML cell lines.

Results: RARα acts as a repressive transcription factor until bound by SY-1425 leading to potent, targeted activation of myeloid genes. HMAs can further prime this activation by depleting repressive methylation of these target genes. The combination of SY-1425 and azacitidine showed synergy in RARA-high AML cell lines, but not in RARA-low AML cell lines, with combination indices less than 0.5. Co-administration in a RARA-high AML PDX demonstrated superior reduction of tumor burden (<1% detectable tumor cells) vs either treatment alone (75% survival in SY-1425 and 8% with azacitidine). Various combination regimens evaluated in the PDX model over two cycles (56 days) found that 1 week of azacitidine followed by 3 weeks of SY-1425 maximized for anti-tumor activity (<5% AML cells in periphery, bone marrow and spleen) and tolerability (<8% weight loss). RARα binds directly to the CD38 locus and induces H3K27 acetylation, a histone modification causing CD38 transcription. Analysis of the mRNA transcripts in RARA-high models. SY-1425 treatment of four RARA-high AML cell lines and three RARA-high primary AML patient samples induced cell surface CD38 to high levels comparable to those of DARA sensitive multiple myeloma cells. In contrast, no CD38 induction was observed in RARA-low cell lines. RARA-high AML cell lines treated with SY-1425 and DARA were six fold more sensitive to NK cell mediated ADCC compared to single agent controls and exhibited a 5-10 fold increase in NK cell-dependent activation measured by IFNγ secretion.

Summary/Conclusions: This RARα biomarker dependent synergy with azacitidine and SY-1425 is hypothesized to work through hypomethylation based priming of myeloid differentiation by SY-1425 agonism of formerly repressed RARα target genes. Since CD38 is one of the most strongly induced RARα target genes in response to SY-1425, AML blasts can be sensitized to DARA in a biomarker dependent manner. The preclinical synergistic effects and anticipated non-overlapping clinical toxicity profiles of the respective agents provide a strong rationale for clinical evaluation of each SY-1425 combination in biomarker selected AML and MDS patients.

FLT3 INHIBITION OVERCOMES RESISTANCE TO THE BCL-2 SELECTIVE ANTAGONIST, VENETOCLAX, IN FLT3-ITD MUTANT AML MODELS

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Background: FLT3 internal tandem duplication (ITD) mutations account for ~20-25% of adult AML cases and are associated with worse prognosis. Although FLT3 inhibitors show clinical activity, relapse occurs quickly. Veneto- clax is a potent, selective inhibitor of the anti-apoptotic protein BCL-2 that demonstrated monotherapy activity in relapsed/refractory AML (ORR 19%); however, no activity was seen in FLT3 mutant cases (Konopleva, Can Disc and ASH 2016). The M14 cell line harbors FLT3-ITD mutations, RARA and RPS. Taken together, our preliminary data suggests that already at diagnosis, AML cells display an epigenomic fingerprint associated with the development of AML relapse during the course of disease. We are currently validating these data.

Summary/Conclusions: Analysis of promoters and especially enhancers is a highly useful approach to identify cell state specific regulation. Here, we analyzed pediatric AML patients at diagnosis and at relapse to gain more insight into specific cell states which are involved in relapse. Our data revealed high similarity between diagnosis and relapse samples, while, strikingly, in the WHO intermediate-risk group containing MLL-rearranged patients, differential epigenomic feature enrichment was observed between NRPs and RPs. Taken together, our preliminary data suggests that the development of AML is regulated in a biomarker dependent manner. The preclinical synergistic effects and antitumor activity of FLT3-ITD in biomarker selected AML and MDS patients (Phase 2 study, NCT02807558). Based on the RARα mediated myeloid gene activation of SY-1425, epigenetic priming with hypomethylating agents (HMAs) and CD38 induction were explored.

Aims: We sought to investigate mechanically informed combinations of SY-1425 and other agents (HMAs and novel agents in AML) and that strong upregulation of the maturation marker CD38 in AML cells by SY-1425 mediated reprogramming by relieving aberrant methylation of RARα target genes and that strong upregulation of the maturation marker CD38 in AML cells by SY-1425 could induce sensitivity to the anti-CD38 therapeutic antibody daratumumab (DARA).

Methods: HMA synergy was tested in vitro in AML cell lines over a range of concentrations for SY-1425 and azacitidine. In vivo studies used a disseminated patient derived xenograft (PDX) model of AML expressing high levels of RARA. SY-1425 induction of CD38 was assessed by H3K27ac/ChiP-seq, RARA ChiP-seq and flow cytometry. Antibody dependent cell-mediated cytotoxicity (ADCC) was tested in an ex vivo co-culture model of human NK cells and AML cell lines.

Results: RARα acts as a repressive transcription factor until bound by SY-1425 leading to potent, targeted activation of myeloid genes. HMAs can further prime this activation by depleting repressive methylation of these target genes. The combination of SY-1425 and azacitidine showed synergy in RARA-high AML cell lines, but not in RARA-low AML cell lines, with combination indices less than 0.5. Co-administration in a RARA-high AML PDX demonstrated superior reduction of tumor burden (<1% detectable tumor cells) vs either treatment alone (75% survival in SY-1425 and 8% with azacitidine). Various combination regimens evaluated in the PDX model over two cycles (56 days) found that 1 week of azacitidine followed by 3 weeks of SY-1425 maximized for anti-tumor activity (<5% AML cells in periphery, bone marrow and spleen) and tolerability (<8% weight loss). RARα binds directly to the CD38 locus and induces H3K27 acetylation, a histone modification causing CD38 transcription. Analysis of the mRNA transcripts in RARA-high models. SY-1425 treatment of four RARA-high AML cell lines and three RARA-high primary AML patient samples induced cell surface CD38 to high levels comparable to those of DARA sensitive multiple myeloma cells. In contrast, no CD38 induction was observed in RARA-low cell lines. RARA-high AML cell lines treated with SY-1425 and DARA were six fold more sensitive to NK cell mediated ADCC compared to single agent controls and exhibited a 5-10 fold increase in NK cell-dependent activation measured by IFNγ secretion.

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decreased expression of IGFBP7 might be associated with decreased chemotherapy sensitivity. To this end, we generated cell lines with IGFBP7 knockdown and subjected the cells to chemotherapy. Furthermore, to test whether increasing the IGFBP7 levels might be a strategy to deplete leukemic (stem) cells, we overexpressed IGFBP7 in or added recombinant human IGFBP7 (rhIGFBP7) to primary AML cells and measured clonogenic capacity, differentiation and cell survival in vitro. To study the effect of IGFBP7 on AML cell survival and engraftment potential in vivo, primary AML cells were transplanted into immune deficient mice and the mice were subsequently treated with rhIGFBP7. To study the effect of rhIGFBP7 on LSC survival, human AML cells derived from the first transplanted mice were re-transplanted into secondary recipients and engraftment and survival of the mice were monitored.

Results: Knockdown of IGFBP7 results in reduced sensitivity to chemotherapy and comparing matched diagnosis and relapsed AML samples showed that IGFBP7 expression is frequently downregulated at relapse, suggesting a survival advantage of IGFBP7+AML cells during chemotherapy treatment. Importantly, enhancing cytoplasmic or extracellular IGFBP7, by overexpression or addition of rhIGFBP7, resulted in induction of differentiation and apoptosis, increased sensitivity to chemotherapy and inhibited AML blast and leukemic stem/progenitor cell survival in vitro and in vivo. IGFBP7 had no influence on the survival of normal hematopoietic (stem) cells. Moreover, treatment with rhIGFBP7 can add to chemotherapy treatment by elimination of chemotherapy resistant refractory AML (stem) cells.

Summary/Conclusions: Altogether, these data suggest that addition of IGFBP7 to the currently used chemotherapy regimens might be a promising strategy to specifically eradicate LSCs and decrease AML relapse rates.

Acute myeloid leukemia - Clinical 1

P191

ONGOING PHASE 2 CLINICAL TRIAL OF SL-401 IN PATIENTS WITH BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM: STAGE 1 AND STAGE 2 RESULTS

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Background: SL-401 is a targeted therapy directed to interleukin-3 receptor α (CD123), a target overexpressed on a variety of cancers including blastic plasmacytoid dendritic cell neoplasm (BPDCN), a highly aggressive malignancy with poor outcomes and unmet medical need.

Aims: This Phase 2 trial is a single-arm, open-label, study designed to generate efficacy and safety data to support potential registration in BPDCN

Methods: In this ongoing Phase 2 single-arm trial, patients with BPDCN (n=32) or relapsed/refractory (R/R) AML (n=48) received SL-401 as a daily IV infusion at 7, 9, 12, or 16 ug/kg/day for days 1-5 of a 21-day cycle in stage 1. In stages 2 and 3, patients received SL-401 at the dose determined in stage 1.

Results: 32 adult BPDCN patients received SL-401 in stage 1 (n=9) and stage 2 (n=23), including 19 first-line and 13 R/R patients. Stage 3 patients will be reported separately. Median age was 72 years (range: 30-85 years). In stage 1, 12 ug/kg was the highest tested dose for BPDCN; MTD was not reached in BPDCN. Median follow-up was 4.3 months (range: 0.25-22.9 months). ORR of 84% (27/32) was observed in all patients; 95% (18/19) in first-line and 69% (9/13) in R/R. 88% (14/16) of first-line patients treated at 12 ug/kg had a complete remission (CR) (n=10), CR with incomplete hematologic recovery (CRI) (n=1) or clinical CR (CRC; residual skin disease) (n=3) based on investigator assessment. 56% (9/16) of these patients were progression free for 4 to 22.9 months (ongoing), including 3 patients on SL-401 in remission (R/R) for 12 months (ongoing) and 7 patients who were bridged to stem cell transplant (SCT; 3 auto-SCT and 4 allo-SCT). A R/R patient was also bridged to allo-SCT. Overall, most common grade 3 treatment-related AEs were transaminase elevation (22%) and thrombocytopenia (16%). Safety precautions, including daily monitoring of albumin and body weight during study drug infusions, have been implemented to minimize risk of severe capillary leak syndrome (CLS). Three patients had Grade 5 CLS: BPDCN (7 ug/kg); R/R AML (16 ug/kg); BPDCN (12 ug/kg) out of 118 patients who received SL-401 across all trials and regimens; 3/89 (3.4%) patients of which were enrolled in this clinical trial.

Summary/Conclusions: SL-401 continues to demonstrate single agent activity, including multiple CRs, in patients with BPDCN, with 25% (8/32) of patients bridged to SCT after a major response from SL-401. SL-401 side effect profile is minimal. Three patients had thrombocytopenia (16%). Safety precautions, including daily monitoring of albumin and body weight during study drug infusions, have been implemented to minimize risk of severe capillary leak syndrome (CLS). Three patients had Grade 5 CLS: BPDCN (7 ug/kg); R/R AML (16 ug/kg); BPDCN (12 ug/kg) out of 118 patients who received SL-401 across all trials and regimens; 3/89 (3.4%) patients of which were enrolled in this clinical trial.

P192

PROGNOSTIC IMPACT OF SOMATIC MUTATION CLEARANCE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Persistence of somatic mutations at the time of complete remission (CR) was associated with poor outcome in patients (pts) with AML.

Aims: To analyze differential pattern of mutation clearance based on the genes and affected pathway and to assess prognostic impact of mutation clearance in AML patients.

Methods: We studied 95 pts with AML who were treated with frontline induction and subsequently achieved CR. We sequenced pre-treatment and CR bone marrow samples by targeted capture sequencing of 295 genes (median 280x coverage). We defined 3 levels of mutation clearance (MC) based on variant allele frequency (VAF): 1) MC2.5, persistent mutation with VAF<2.5%, 2) MC1.0, persistent mutation with VAF<1%, and 3) complete mutation clearance (CMC).

Results: In the pre-treatment samples, we detected 597 mutations in 78 genes in 87 (92%) patients. In the matching CR samples, 62 (10%) and 82 (14%) mutations persisted at VAF>2.5% and ≥1%, respectively, which corresponded to 43 (49%), 34 (39%), and 30 (34%) patients achieving MC2.5, MC1.0 and CMC, respectively. Table 1 shows the differential patterns of MC based on the mutations and pathways. Mutations associated with clonal hematopoiesis of
indeterminate therapy (CHIP), DNA methylation, and splicing pathways had low rate of MC, whereas mutations in transcription factors, receptor tyrosine kinase (RTK) had high rate of MC. Pts who achieved MC1.0 (median 31.2 vs 12.5 months, P=0.04) or CMC (median 31.2 vs 12.5 months, P=0.049) had significantly better relapse-free survival (RFS).

Table 1.

<table>
<thead>
<tr>
<th>Gene</th>
<th>MC1.0 (%)</th>
<th>CMC (%)</th>
<th>Pathway</th>
<th>MC2.0 (%)</th>
<th>CMC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHOP</td>
<td>21%</td>
<td>17%</td>
<td>CHIP</td>
<td>33%</td>
<td>24%</td>
</tr>
<tr>
<td>FLT3</td>
<td>100%</td>
<td>99%</td>
<td>CHIP</td>
<td>39%</td>
<td>29%</td>
</tr>
<tr>
<td>TET2</td>
<td>15%</td>
<td>13%</td>
<td>RTK</td>
<td>88%</td>
<td>87%</td>
</tr>
<tr>
<td>TAL1</td>
<td>100%</td>
<td>99%</td>
<td>CHIP</td>
<td>89%</td>
<td>89%</td>
</tr>
<tr>
<td>CEBPA</td>
<td>100%</td>
<td>99%</td>
<td>CHIP</td>
<td>67%</td>
<td>55%</td>
</tr>
<tr>
<td>IDH2</td>
<td>38%</td>
<td>34%</td>
<td>Splicing</td>
<td>33%</td>
<td>17%</td>
</tr>
</tbody>
</table>

Summary/Conclusions: Somatic mutations associated with CHIP, DNA methylation, and splicing pathways persisted frequently in CR samples suggesting preleukemic origin. Pts with deeper MC had significantly better RFS. Somatic mutation clearance may help risk prediction of AML.

P193

DO EDUCATION AND INCOME AFFECT TREATMENT AND OUTCOME IN ACUTE MYELOID LEUKEMIA IN A TAX-SUPPORTED HEALTH CARE SYSTEM? A DANISH NATIONAL POPULATION-BASED COHORT STUDY

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Background: No larger study has investigated the association between individual-level education or income level and clinical prognostic markers, treatment, and outcome in acute myeloid leukemia (AML). Understanding how socioeconomic status (SES) affects survival in AML patients may improve prognostication through targeted support among patients with different SES risk profiles. Aim: We investigate the effects of education as a knowledge-related SES factor and income as a measure of material resources in a tax-supported health care system linking individual-level SE information from Statistics Denmark to clinical data from the Danish National Leukemia Registry.

Methods: We conducted a nationwide population-based cohort study and included AML patients ≥25 years diagnosed in Denmark 2000-2014 (end of follow-up, Feb 2016). KM curves and Cox regression (Hazard ratios; HRs) was included AML patients ≥25 years diagnosed in Denmark 2000-2014 (end of follow-up, Feb 2016). KM curves and Cox regression (Hazard ratios; HRs) was used to compare survival by education (low, medium, and high) and income level (tertiles). We repeated the survival analysis within educational groups by years of diagnosis (2000-2004, 2005-2009, 2010-2014), stratified by time period, and calculated crude survival (%) at 1, 3, and 5 years. We used logistic regression (odds ratios; ORs) to compare treatment intensity, chance of clinical trial inclusion, and complete remission (CR) between groups. Results were given stratified by age (<60/≥60 years). Results: Of 2992 patients, 1588 (53.1%) received remission induction chemotherapy. Forty-five percent (n=1336) completed a low-level education, 38% (n=1138) a medium education, and 17.3% (n=518) a higher education. Patients with higher education tended to be younger and to be male. In intensive therapy patients <60 years, survival was superior in high-education patients evident a year from diagnosis (1-year survival: high 65.2%, HR 1.0, medium 59.2%, adjusted HR 1.55 [CI=1.21-1.98], low 57.7%, 1.47 [CI=1.11-1.93]). Allongenic transplantation rates in CR1 were significantly higher in high-education compared with low-education patients (16.3% versus 8.7%). Overall survival in high-educated patients improved over time: HR 0.78 (CI=0.61-0.99), medium 0.99 (CI=0.84-1.16), and low 1.03 (CI=0.84-1.27) increasing the survival gap between educational groups (Low: year 2000-2004 HR 1.28 [CI=0.88-1.85], 2004-2009 HR 1.51 [CI=0.61-4.77] and 2010-2014 HR 2.09 [CI=1.27-3.44], high 1.0); Figure 1. In older patients, low education was associated with lower chance of intensive therapy (30% versus 48%; adjusted OR 0.65 [CI=0.44-0.98] compared to high-education, however neither CR rates in intensive therapy patients nor survival overall or in intensive therapy patients was affected. Low-income patients where less likely to be enrolled in clinical trials (low-income 22.8%, adjusted OR 0.55 [CI=0.39-0.79] medium 28.2%, adjusted OR 0.71 [CI=0.53-0.94]) compared to high-income (37.2%, HR 1.0), however, income was not associated with therapy intensity, chance of CR, or survival (intensive therapy-only; high income adjusted HR 1.0, medium 0.96 [CI=0.82-1.12], low 1.06 [CI=0.88-1.27]).

Summary/Conclusions: In Denmark where health-care is free and uniform, high SE status does not affect treatment intensity in younger patients or response to therapy. However, educational level, but not income, influences all-cause mortality and has a major impact on survival in younger AML patients. Since 2000, survival improvements have exclusively benefitted well-educated patients and additional attention during treatment and follow-up towards low-educated patients may increase transplantation rates and improve survival.

P194

IDENTIFICATION OF PATTERNS IN CO-OCCURRING MUTATIONS IN AML PATIENTS WITH GERMLINE AND SOMATIC RUNX1 MUTATIONS

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Background: RUNX1 plays a vital role in leukemogenesis through its interaction with core binding factor-β complex and other genes involved in hematopoiesis (1,2). Familial platelet disorder with predisposition to acute myeloid leukemia (FPD/AML) is linked to germline RUNX1 mutations (3). This autosomal dominant disorder is characterized by thrombocytopenia and potential for transformation to AML. AML patients with somatic RUNX1 mutations have a poor prognosis (6,7) independent of other risk factors. The role of co-occurring mutations in leukemogenesis in FPD/AML patients with germline RUNX1 mutations and AML patients with de novo somatic RUNXI mutations is not fully understood.

Aims: In order to further characterize co-occurring mutations in patients with both germline and somatic RUNX1 mutations, we analyzed a large cohort of AML tumor samples along with several paired normal tissue samples.

Methods: We sequenced a cohort of 482 diagnostic bone marrow or peripheral blood samples from AML patients by deep whole-exome sequencing. Samples were collected through the “Beat AML” project, an ongoing program at Oregon Health & Science University in collaboration with the Leukemia & Lymphoma Society. RUNXI mutations were classified using VarScan which defined somatic and germline mutations as follows: somatic if p<0.1 and germline if not called as somatic and normal variant allele frequency >0.1.

Results: Twenty AML samples had 21 germline RUNX1 mutations with a total of 91 different germline variants; 31 other patient samples had 38 somatic RUNX1 mutations with 31 unique somatic variants. One sample had 2 RUNX1 germline mutations; 6 samples had >1 somatic RUNX1 mutations. The most common germline variant, missense mutation p.L56S, was found in 16 (76%) of 21 germline mutations, 16 had co-occurring known pathogenic mutations in AML-related genes. Most significantly, 62% (10/16) and 51% (14/27) of patients with germline or somatic RUNX1 mutations, respectively, had 7 co-occurring AML-related pathogenic mutations that were exclusive to their cohort (Table 1). Both germline and somatic RUNX1 mutational cohorts had 12 overlapping co-occurring mutations. The most common mutations, for both groups, were in FLT3 (14/43), ASXL1 (8/43), and IDH2 (7/43) (Table 1). Patient demographics and treatment-related outcomes were similar for both cohorts.

Figure 1.

Summary/Conclusions: In Denmark where health-care is free and uniform, high SE status does not affect treatment intensity in younger patients or response to therapy. However, educational level, but not income, influences all-cause mortality and has a major impact on survival in younger AML patients. Since 2000, survival improvements have exclusively benefitted well-educated patients and additional attention during treatment and follow-up towards low-educated patients may increase transplantation rates and improve survival.
Methods: Ninety consecutive patients diagnosed with de novo AML at our institution and eligible for intensive chemotherapy were enrolled from September 2010 to March 2016. We excluded 10 patients with acute promyelocytic leukemia. This study was approved by the institutional review board of the Ethics Committee and the primary endpoint was overall survival (OS). The secondary endpoint was progression-free survival (PFS). OS and PFS were estimated using the Kaplan-Meier method, and assessed using the log-rank test. Multivariate analysis using Cox proportional hazard ratio was performed for OS and PFS.

Results: The median follow-up for patients still alive at the end of the study was 38.9 months (range: 1.5-64.8 months). The median patient age was 60 years (range: 17-78 years). There was no statistical significance between LSCHigh patients (n=30) and LSCLow patients (n=50) in sex, age, laboratory data, NPM1 mutation, or European Leukemia Net karyotype risk group. FLT3 mutation was associated with the LSCHigh group (p=0.003). Three-year OS and PFS were significantly better in the LSCHigh group than in the LSCLow group (Figure 1) (OS: 65.0% vs 18.2%, p < 0.001; PFS: 49.3% vs 19.4%, p < 0.001). In multivariate analysis controlled for age and karyotype (Table 1), being in the LSCHigh group was an independent prognostic factor for OS (hazard ratio: 3.17; 95% CI: 1.64-6.15; p < 0.001) and PFS (hazard ratio: 2.25; 95% CI: 1.24-4.08; p=0.007). Being in the LSCHigh group had incremental value for OS compared with the karyotype risk (Harrell's C index: 0.80 vs 0.70; p = 0.028). Moreover, this classification based on LSC marker expression allowed subgroups with unfavorable prognosis to be identified among patients in the intermediate karyotype risk group (3y-OS 54.6% vs 14.5%, p=0.013), as well as those in the favorable karyotype risk group (3y-OS 94.1% vs 50.0%, p=0.021).

Summary/Conclusions: The incidence of RUNX1 mutations seen in our 482-patient Beat AML cohort (4.3% germline, 6.4% somatic) is consistent with results from other studies (8). Our study suggests that germline and somatic RUNX1 mutations in AML patients are mutually exclusive, as are several co-occurring pathogenic mutations that contribute to leukemogenesis. Our study adds to the already described mutually exclusive mutations in germline RUNX1 by identifying WT1, CHEK2, CCND3, and others. Similarly, in samples with somatic RUNX1 mutations, we found mutually exclusive mutations in CBL, JAK2, MLL, EZH2 and others, in addition to the previously described IDH1 (8). Further characterization of these results and analyses of additional samples using our whole-exome sequencing and our bioinformatics platform will help us better elucidate the molecular events underlying AML progression and help us establish novel prognostic/therapeutic markers aimed at early intervention in patients, or their family members, who carry RUNX1 mutations.

P195

Abstract withdrawn.

P196

MULTIPLE LEUKEMIC STEM CELL MARKER EXPRESSION IS ASSOCIATED WITH POOR PROGNOSIS IN DE NOVO ACUTE MYELOID LEUKEMIA: T. Yabushita1*, A. Matsushita1, H. Hashimoto2, T. Ishikawa1

Background: Acute myeloid leukemia (AML) is believed to originate from a small population of leukemic stem cells (LSCs). Current chemotherapy regimens target the majority of more mature leukemic blasts, but cannot efficiently eliminate LSCs, resulting in early treatment failure and relapse. Thus, the expression of LSC-specific markers could be used as a predictive factor of clinical outcomes in AML patients. Recently, the clinical impact of individual LSC markers has been documented in several reports, but the combined effect of different LSC markers remains unexamined.

Aims: This study aimed to estimate the prognostic impact of the expression of multiple LSC markers on the outcome of AML patients.

Methods: Ninety consecutive patients diagnosed with de novo AML at our institution and eligible for intensive chemotherapy were enrolled from September 2010 to March 2016. We excluded 10 patients with acute promyelocytic leukemia. This study was approved by the institutional review board of the Ethics Committee and compliant with the Declaration of Helsinki. We analyzed the expression of three LSC markers, CD25, CD96, and CD123, in de novo AML patients. The expression of these markers on gated leukemic blasts was evaluated using 6-color flow cytometry. When over 20% of leukemic blasts were positive for any marker, the sample was defined as positive for that marker. We stratified de novo AML patients into two groups: LSCHigh was defined as positivity for two or three LSC markers, and LSCLow was defined as negativity for all markers or positivity for a single LSC marker. The primary endpoint was overall survival (OS). The secondary endpoint was progression-free survival (PFS). OS and PFS were estimated using the Kaplan-Meier method, and assessed using the log-rank test. Multivariate analysis using Cox proportional hazard ratio was performed for OS and PFS.

Results: The median follow-up for patients still alive at the end of the study was 38.9 months (range: 1.5-64.8 months). The median patient age was 60 years (range: 17-78 years). There was no statistical significance between LSCHigh patients (n=30) and LSCLow patients (n=50) in sex, age, laboratory data, NPM1 mutation, or European Leukemia Net karyotype risk group. FLT3 mutation was associated with the LSCHigh group (p=0.003). Three-year OS and PFS were significantly better in the LSCHigh group than in the LSCLow group (Figure 1) (OS: 65.0% vs 18.2%, p < 0.001; PFS: 49.3% vs 19.4%, p < 0.001). In multivariate analysis controlled for age and karyotype (Table 1), being in the LSCHigh group was an independent prognostic factor for OS (hazard ratio: 3.17; 95% CI: 1.64-6.15; p < 0.001) and PFS (hazard ratio: 2.25; 95% CI: 1.24-4.08; p=0.007). Being in the LSCHigh group had incremental value for OS compared with the karyotype risk (Harrell's C index: 0.80 vs 0.70; p = 0.028). Moreover, this classification based on LSC marker expression allowed subgroups with unfavorable prognosis to be identified among patients in the intermediate karyotype risk group (3y-OS 54.6% vs 14.5%, p=0.013), as well as those in the favorable karyotype risk group (3y-OS 94.1% vs 50.0%, p=0.021).

Summary/Conclusions: We demonstrated that multiple LSC marker expression predicts poor clinical outcomes in newly diagnosed de novo AML patients, and may facilitate better stratification even among patients with intermediate-risk and favorable-risk karyotypes.

P197

NEXT GENERATION SEQUENCING TARGETED PANEL FOR MINIMAL RESIDUAL DISEASE MONITORING IN ACUTE MYELOID LEUKEMIA: V. Mcclain1*, A.R. Carson1, B.A. Patay1, L. Chamberlain 1, C. Chander 1, S. Zheng 1, W. Huang 1, O. Kiya 1, D. Hubbard 2, D. Caguioa 2, Z. Xie 1, V. Mcclain1*, A.R. Carson 1, B.A. Patay 1, L. Chamberlain 1, C. Chander 1, S. Zheng 1, W. Huang 1, O. Kiya 1, D. Hubbard 2, D. Caguioa 2, Z. Xie 1, J. Thrones 1, T. Stenzel 1, J. E. Miller 1, Invisascience, LabPMM LLC, San Diego, United States

Background: Many personalized therapies for acute myeloid leukemia (AML) have been developed targeting specific biomarkers. Unfortunately, the efficacies of these therapies are inconsistent while the need to determine successful therapies prior to patient relapse is critical. Minimal residual disease (MRD) monitoring can help determine effective treatments and predict potential relapse. While there are now several MRD tests available on the market, most target single or small numbers of biomarkers, which can limit detection of residual AML heterogeneity. Thus, full characterization of a sample may require testing with multiple MRD assays, which can be impractical in a clinical setting. We have developed a target capture-based assay (MyMRD™), which allows characterization of the entire therapeutic AML biomarker repertoire and can inform full characterization of the entire therapeutic AML biomarker repertoire and can inform...
the molecular remission status of a patient’s malignancy. This targeted panel can identify the mutations in driver clones that cause relapse in ~90% of all AML patients, as well as common drivers in myeloid proliferative neoplasms (MPN) and myelodysplastic syndromes (MDS).

**Aims:** To establish a sensitive and reliable targeted NGS assay to comprehensively detect and monitor the majority of known driver mutations in AML and MDS cases.

**Methods:** Whole genome libraries, made from DNA extracted from cell lines and clinical samples, were hybridized with MyMRD probes targeting mutation hotspots in 23 genes associated with AML. In addition to single nucleotide variants (SNVs) and indels, we also targeted structural variants (SVs) in 21 of these genes. Cases showing two variants were further analyzed using proprietary Invivoscribe (IVS) MyInformatics<sup>TM</sup> software. To validate mutations detected by the MyMRD assay, samples were additionally tested with IVS developed capillary electrophoresis (CE) assays and NGS-based assays targeting common mutations in FLT3 and NPM1.

**Results:** The linearity and limit of detection (LOD) of the MyMRD assay were assessed using data generated from contrived cell line DNA containing known AML driver mutations with a range of variant allele frequencies (VAFs). The assay shows strong linearity (R²=0.96 – 0.99) in the entire range of tested VAFs (0.01% – 0.2%). Overall, we established a LOD of 0.5% for >95% of the targeted sites in the assay with lower LODs for specific mutations of interest (e.g. 0.1% for a 30bp FLT3 ITD and 0.2% for FLT3 p.D835Y). Additionally, specific mutations of interest, such as those used for residual disease monitoring (e.g. FLT3 ITD), demonstrate LODs as low as 0.1%. The MyMRD assay provides an accurate method for detecting mutations in multiple targets in patients and can be used to effectively stratify patients for therapy and clinical trials.

**Figure 1.**

**Summary/Conclusions:** Since AML patients with biCEBPA mutations have relatively favourable overall survival, it is important in the clinical setting to accurately assess CEBPA molecular status. In our study, we have tested the ability of three different assays to detect CEBPA mutations in 173 samples. Sanger sequencing was the only method actually covering the entire coding region of CEBPA. Both NGS ampiclon-based panels failed to fully cover the coding region of the gene, and therefore have likely missed mutations. Crucially, even when any of the three methods detected more than one variant, cloning studies confirmed biCEBPA mutations only in a fraction of the cases. In summary, none of the amplicon-based tested methods can reliably determine if multiple mutations affect two different alleles; therefore biCEBPA mutations would still need additional confirmation. We are currently exploring the ability of capture-based NGS approaches coupled to appropriately tailored bioinformatic analysis of sequencing data to detect biCEBPA mutations.
(18-83 years). All patients were treated with standard induction and consolidation protocols.

Results: Median time between two investigations was 2.8 months (range for all 0.1-115 months). A complete molecular remission was reached in 90/130 pts (69%) after a median of 5 months. 19/130 (14.6%) pts reached low level MRD and 20/130 (15.4%) high level MRD. Median event free survival (EFS) of patients with CMR was not reached (EFS at 2 years 82%). 16 (18%) of those patients relapsed in the course of follow up with a median time to relapse of 12.7 months (range 4.1 to 38.3 months). Median EFS for MRD low and MRD high patients was 18.4 months and 10.8 months respectively (all 3 groups, p<0.0001). For patients with CMR, rising MRD levels accurately predicted relapse with a median latency of 5.5 months from loss of CMR to relapse. We next used the widely accepted log fold change from baseline to define high and low risk patients in our cohort. 123/130 (95%) patients reached a >3 log fold reduction in RUNX1-RUNX1T1/ABL ratio within the first 200 days following first diagnosis. Median EFS for those patients was not reached (EFS at 2 years 66%). The 7/130 (5%) patients with a <3 log fold reduction had a median EFS of 14.7 months (2 groups, p=0.017). A total of 59/185 patients received allogeneic SCT. Among the 130 patients diagnosed at our laboratory 34 (26%) received allogeneic SCT, 12 (9%) were transplanted in first CR and 17 (13%) were transplanted for relapse. Following allogeneic SCT 11/17 patients (65%) reached a second CR with CMR.

Summary/Conclusions: Our data shows that MRD testing is routinely performed in RUNX1-RUNX1T1 AML outside of clinical studies. Defining MRD levels by RUNX1-RUNX1T1/ABL ratios resulted in a better classifier for high and low risk patients than log fold change. However, despite CMR 16/90 (18%) patients relapsed with a maximum time from first achievement of CMR of 38.3 months. We conclude that 1) MRD monitoring could serve to guide BMT decisions in RUNX1-RUNX1T1 positive AML, 2) allogeneic BMT can rescue the majority of relapsed patients and 3) molecular monitoring can reliably identify patients with high risk for relapse.
Acute myeloid leukemia - Clinical 2

P200

NUMEROUS TP53 ABNORMALITIES AND THEIR CLINICAL RELEVANCE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA AND MYELODYSPLASTIC SYNDROMES

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Background: Mutations in TP53 can be detected in up to 16-19% of patients with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). TP53 mutations confer adverse prognosis irrespective of currently available therapies. The clinical impact of the type and number of TP53 abnormalities is unclear.

Aims: To evaluate the prognostic impact of the number of TP53 abnormalities in AML and MDS.

Methods: We evaluated 1401 patients with previously untreated AML or MDS treated at the University of Texas MD Anderson Cancer Center from 2012 to 2016. Sequencing data was obtained by use of a 28 or 53-gene targeted PCR-based next generation sequencing platform. Response was defined following 2003 IWG criteria for patients with AML and 2006 revised IWG criteria for patients with MDS. Generalized linear models were used to study the association of overall response (OR), complete response (CR) and risk factors, Kaplan-Meier produce limit method was used to estimate the median overall survival (OS).

Results: A total of 593 (42%) patients had MDS and 808 (56%) had AML. In a total of 984 (70%) patients, data on therapy with sufficient follow up and response evaluation was available, with 494 (35%) patients receiving therapy with hypomethylating agents (HMAs) and 373 (27%) with chemotherapy regimens. A total of 384 mutations in TP53, involving 208 unique mutations, were detected among 300 (21%) patients with R273H, R248W, V220C and R175H being the most prevalent. Overall frequency of TP53 mutations was higher among patients with MDS (25%, n=146) compared to AML (19%, n=154) (p=0.012) with 251 (84%) of detected mutations happening in patients with complex karyotype (p=0.001). Among patients with TP53-mutant disease, 221 (74%) had 1 detectable mutation, 76 (25%) had 2 and 3 (1%) had 3. Additionally, 188 (13%) patients had TP53 deletions evidenced by presence of monosomy 17 or del(17p). In 167 (89%) of these patients, chr17 abnormalities were detected in the context of a complex karyotype and in 127 (42%) a co-occurring TP53 abnormality, 169 (12%) with TP53 mutations and deletions (p=0.443, p<0.001) was observed with 172 (12%) patients having 1 TP53 abnormality, 169 (12%) having 2 detectable TP53 mutations were less likely to have co-occurring chr17 abnormalities (79% vs 22%, OR 0.28, CI 0.15-0.50, p=0.03). Median follow up was 8.6 months (range 0-167 months). Presence of a TP53 mutation adversely impacted OS (MDS: 12 vs 111.7 months, HR=5.98, CI 4.28-8.33, p<0.001; AML: 5.3 vs 16.9 months, HR=2.81, CI 2.26-3.50, p<0.001). Increasing number of TP53 abnormalities negatively impacted OS of patients with AML (Figure 1A) but not that of patients with MDS (Figure 1B). No difference in survival was observed between patients with two TP53 mutations and those with TP53 mutation+deletion (p=0.730). Presence and number of TP53 mutations did not predict for response (OR: 60 vs 63% p=0.498; CR: 34 vs 36%, p=0.695) to HMAs, but was associated with significantly lower likelihood of response to intensive chemotherapy (OR: 41 vs 86%, p<0.001; CR: 33 vs 75%, p<0.001).

Summary/Conclusions: Presence of multiple TP53 abnormalities can be observed in up to 13% patients with AML and MDS. Second TP53 abnormalities more commonly involve TP53 deletions with additional TP53 mutations being less common and generally mutually exclusive with TP53 deletions. The number of TP53 abnormalities impacts the survival of patients with AML but not that of patients with MDS. Presence and number of TP53 mutations do not seem to impact response to HMAs but are associated with lower responses to chemotherapy.

P201

VADASTUXIMAB TALIRINE PLUS HYPMETHYLATING AGENTS: A WELL-TOLERATED REGIMEN WITH HIGH REMISSION RATE IN FRONTLINE OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA- FISH + TP53- MUTATION POSITIVE: FISH-POSITIVE BONE MARROW ABNORMALITIES IMPACTS THE SURVIVAL OF PATIENTS WITH AML BUT NOT MDS.

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Background: Treatment of AML among the elderly is challenging. HMAs are commonly used, but yield suboptimal response rates and modest survival. Different regimens were difficult to achieve; in a study of MRD response by flow cytometry in patients treated with single-agent HMA therapy at MD Anderson Cancer Center, only 13/38 (22%) responding patients achieved minimal residual disease (MRD) negativity (F Ravandi, MD, unpublished data, January 2017). Vadastuximab talirine (2GM-C033A; 33A) is a CD33-directed antibody conjugated to 2 molecules of a pyrrolobenzodiazepine (PBD) dimer. Upon binding, 33A is internalized and transported to the lysosomes where PBD dimer is released via proteolytic cleavage of the linker, crosslinking DNA, and leading to cell death.

Aims: A short in a phase 1 study (NCT01902329) was designed to evaluate the safety, tolerability, PK, and antileukemic activity of 33A in combination with an HMA.

Methods: Eligible patients (ECOG status 0-1) had previously untreated CD33-positive AML. One dose of 33A (10 mcg/kg) was administered outpatient IV every 4 weeks on the last day of HMA (azacitidine or decitabine [5-day regimens], standard dosing). CR required either plateau level of ≥100,000/µL or neutrophils of ≥1,000/µL (Cheson 2003). MRD was measured by multiparameter flow cytometry.

Results: Fifty-three patients (median age 75 years [range, 60-87]) were treated (31% females). Patients had advanced disease (38%) or intermediate-risk cytogenic (per MRC); patients were either unfit for (40; 75%) or declined (13; 25%) intensive therapy. The median treatment duration is currently 19.3 weeks (range, 2-86) with 8 patients still on treatment; no DLTs were reported. Adverse events (AEs) ≥grade 3 reported in ≥15% of patients were thrombocytopenia (46%), febrile neutropenia (49%), anemia (46%), neutropenia (42%), pneumonia (19%), and leukopenia (17%); no ≥grade 4 bleeding events were observed. Treatment-emergent (TE) liver lab elevations (≥grade 3) were rare: ALT (8%), AST (2%), and total bilirubin (2%). Other non-heme TEAEs reported in ≥25% of patients regardless of relationship to study treatment were fatigue (60%), nausea (49%), constipation (43%), peripheral edema (38%), anorexia (23%), decreased appetite (40%), dyspnea (34%), pyrexia (32%), diarrhea, vomiting (28%) and dizziness (26%). Thirty- and 60-day mortality rates were 2% and 8%, respectively, with no treatment-related deaths reported. A total of 39 (103/263) of doses were delayed due to AEs mostly from myelosuppression (neutropenia 18%, thrombocytopenia 7%, and febrile neutropenia 3%), high remission rates (37/49 (76% CR+CRI) were maintained across adverse disease subsets including adverse cytogenetics (16/18, 89%), TP53-mutated (56/87, 66%), secondary AML (18/22, 82%), and age ≥75 years (18/26, 69%). Of all responding patients, 19/37 (51%) achieved MRD negativity. Two patients went on to subsequent allo-SCT, and no HMA and HMA/WBCT was observed. The median disease-free survival was 9.1 months (range, 0.1-19.4+). OS continues to evolve with 15 patients (28%) alive (11.3 month median follow-up) (Figure 1).

Summary/Conclusions: 33A+HMA is well tolerated with a safety profile consistent with on-target myelosuppression. The CR+CRI rate of 76% and low early mortality in older AML patients with poor risk factors is particularly encouraging, and activity appears markedly improved compared to the historical experience of HMA monotherapy. The MRD clearance rate among responding patients who received 33A+HMA is higher than the rate observed with single
Figure 1.

P202

ACUTE MYELOID LEUKEMIA WITH INTERMEDIATE-RISK CYTOGENETICS AND A FAVORABLE GENOTYPE: PROGNOSTIC FACTORS AND RESULTS IN PATIENTS TREATED ACCORDING THE SPANISH CETLAM PROTOCOLS

Background: Acute myeloid leukemia (AML) with intermediate-risk (IR) cyto- genetics includes a substantial proportion of patients with favorable molecular profile (FMP); in which AML cells harbor the NPM1 mutation or CEBPA biallelic mutation without internal tandem duplication of the FLT3 gene (FLT3-ITD). The role of allogeneic hematopoietic transplantation (allo-HCT) in first complete remission (CR) in these patients remains controversial.

Aims: To analyze the results and prognostic factors of FMP AML patients in a large series of patients treated by the Spanish CETLAM group.

Methods: Patients with primary AML diagnosed at 19 institutions from the Spanish CETLAM group and treated between 2003 and 2017. Induction chemotherapy included idarubicin and cytarabine (standard or intermediate-dose) in all cases, consolidation with intermediate or high-dose cytarabine (HDAC) and, depending on the protocol, additional HDAC, autologous or allo- geneic hematopoietic transplantation.

Results: Two hundred twenty-one patients were analyzed. Median age of the series was 54 years (range 18 to 72). 152 patients had an age up to 60 years and 69 (31%) were older. Median WBC count was 19x10^9/l (range 0.5-582). One hundred eighty-two patients had a normal karyotype and it was abnormal in 34 (5 patients no metaphases). One hundred ninety-one patients had NPM1 mutated and FLT3-ITD wild type (NPM1+/FLT3-ITD-) and was abnormal in 34 (5 patients no metaphases). One hundred ninety-one patients had an age up to 60 years and 69 (31%) were older. Median WBC count was 19x10^9/l (range 0.5-582). One hundred eighty-two patients had a normal karyotype and it was abnormal in 34 (5 patients no metaphases). One hundred ninety-one patients had NPM1 mutated and FLT3-ITD wild type (NPM1+/FLT3-ITD-) and was abnormal in 34 (5 patients no metaphases).

Figure 1.

Summary/Conclusions: Patients with primary AML, IR cytogenticitics and FMP have a good outcome. Best results are achieved in patients with CEBPα+/FLT3-ITD-, particularly if age is up to 60 years. In this subset, OS at 8 years is 96±7%, comparable to current results achieved in acute promye- locytic leukemia. Patients above 60 years treated intensively may achieve a long term survival of more than 50%. Chemotherapy without subsequent transplantation is a valid option. MRD monitoring after treatment has to be taken into account since in the subset of patients analyzed this was an independent prognostic factor for EFS.

P203

GMI-1271, A POTENT E-SELECTIN ANTAGONIST, COMBINED WITH INDUCTION CHEMOTHERAPY IN ELDERLY PATIENTS WITH UNTREATED AML: A NOVEL, WELL-TOLERATED REGIMEN WITH A HIGH REMISSION RATE

Background: The outcomes for elderly patients (pts) with acute myeloid leukemia (AML) remain poor due to limited tolerance of intensive cytoxic chemotherapy and low response rate, therefore newer and less toxic therapies are urgently needed. The binding of E-selectin (E-sel), an adhesion molecule expressed in the vasculature of the bone marrow, to the leukemic cell surface activates survival pathways and promotes chemotheraphy resistance. GMI-1271, a novel E-sel antagonist, disrupts these survival pathways and enhances antileukemic activity of GMI-1271.

Aims: To analyze the results and prognostic factors of FMP AML patients in a large series of patients treated by the Spanish CETLAM group.

Methods: Patients with primary AML diagnosed at 19 institutions from the Spanish CETLAM group and treated between 2003 and 2017. Induction chemotherapy included idarubicin and cytarabine (standard or intermediate-dose) in all cases, consolidation with intermediate or high-dose cytarabine (HDAC) and, depending on the protocol, additional HDAC, autologous or allo- geneic hematopoietic transplantation.

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P203

GMI-1271, A POTENT E-SELECTIN ANTAGONIST, COMBINED WITH INDUCTION CHEMOTHERAPY IN ELDERLY PATIENTS WITH UNTREATED AML: A NOVEL, WELL-TOLERATED REGIMEN WITH A HIGH REMISSION RATE

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Background: The outcomes for elderly patients (pts) with acute myeloid leukemia (AML) remain poor due to limited tolerance of intensive cytotoxic chemotherapy and low response rate, therefore newer and less toxic therapies are urgently needed. The binding of E-selectin (E-sel), an adhesion molecule expressed in the vasculature of the bone marrow, to the leukemic cell surface activates survival pathways and promotes chemotherapy resistance. GMI-1271, a novel E-sel antagonist, disrupts these survival pathways and enhances antileukemic activity of GMI-1271.

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Results: Two hundred twenty-one patients were analyzed. Median age of the series was 54 years (range 18 to 72). 152 patients had an age up to 60 years and 69 (31%) were older. Median WBC count was 19x10^9/l (range 0.5-582). One hundred eighty-two patients had a normal karyotype and it was abnormal in 34 (5 patients no metaphases). One hundred ninety-one patients had NPM1 mutated and FLT3-ITD wild type (NPM1+/FLT3-ITD-) and was abnormal in 34 (5 patients no metaphases). One hundred ninety-one patients had NPM1 mutated and FLT3-ITD wild type (NPM1+/FLT3-ITD-) and was abnormal in 34 (5 patients no metaphases).

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Summary/Conclusions: Patients with primary AML, IR cytogenticitics and FMP have a good outcome. Best results are achieved in patients with CEBPα+/FLT3-ITD-, particularly if age is up to 60 years. In this subset, OS at 8 years is 96±7%, comparable to current results achieved in acute promye- locytic leukemia. Patients above 60 years treated intensively may achieve a long term survival of more than 50%. Chemotherapy without subsequent transplantation is a valid option. MRD monitoring after treatment has to be taken into account since in the subset of patients analyzed this was an independent prognostic factor for EFS.
Results: 24 pts have been enrolled to date and 17 are evaluable for response. The median age was 69 years (range, 40-79) with 58% male pts and 25% with high-risk cytogenetics (by SWOG). 50% (12/24) were pts with secondary AML (sAML), half of whom had prior hypomethylating therapy (50%; 6/12). This study had a rolling safety run-in and the first 3 pts had no DLT, allowing enrollment to proceed. Common Gr 3/4 AEs included febrile neutropenia (47%), pneumonia (20%), cardiac disease (13%) and non-fatal respiratory failure (13%). 2 pts died of sepsis within 60 days. The remission rate (CR/CRi) was 12/17 (71%). CR/CRi rate was 75% for pts with de novo disease and 67% for pts with sAML. The PK profile in this elderly population was consistent with that of younger adults (median age <60 years) with AML treated with the same dose of glasdegib. Enrollment is planned. A randomized trial is being planned.

Summary/Conclusions: The addition of a novel E-selectin antagonist, GMI-1271, to anthracycline-based induction chemotherapy in untreated elderly pts with AML, including patients with secondary AML, demonstrates a high remission rate with acceptable side effect profile resulting in low induction mortality. This study compares favorably to previous studies (Lancet, ASCO 2016). E-selectin ligand was expressed on leukemic blasts in the majority of pts, therefore supporting its relevance as a target. A randomized trial is being planned.

P204
A PHASE 2 STUDY OF GLASDEGIB (PF-04449913) IN COMBINATION WITH CYTARABINE AND DAUNORUBICIN IN UNTREATED PATIENTS WITH ACUTE MYELOID LEUKEMIA OR HIGH-RISK MYELODYSPLASTIC SYNDROME
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Background: Glasdegib, a selective, once-daily (QD), oral Smoothened (SMO) inhibitor, demonstrated significant improvement in overall survival (OS) when used in combination with low-dose cytarabine (LDAC) vs LDAC alone in a randomized (2:1) open-label trial in 132 patients (pts) not suitable for induction chemotherapy (IC). Preclinical studies showed that glasdegib limits leukemia stem cell proliferation and provided evidence of glasdegib synergy with chemotherapy.

Aims: Primary objective of this open-label, single-arm Ph 2 study (NCT01546038) was to determine complete remission (CR) rate with glasdegib in combination with cytarabine and daunorubicin in untreated AML or high-risk MDS pts. OS was the key secondary endpoint.

Methods: Pts suitable for ICT (ECOG PS 0-1, creatinine ≤1.3 mg/dL, no severe cardiac disease) gave informed consent and received glasdegib 100 mg QD from day -3 in combination with cytarabine 100 mg/m² CI for 7 days and daunorubicin 60 mg/m² IV for 3 days, followed by 2-4 consolidation cycles (cytarabine 1 gm/m² 2 hrs on days 1, 3, 5). Maintenance (up to 6 months) included glasdegib 100 mg QD. Pts were assessed for efficacy, safety and tolerability.

Results: All Pts: As of 1 Dec 2016, 71 pts (66 AML, 5 MDS) were enrolled and 69 pts received glasdegib and ICT (2 pts not treated due to ineligibility). Among the 19 secondary AML pts, 20% had favorable, 32% INT, intermediate (int)-II, 21% int-III and 26% adverse cytogenetic abnormalities (by SWOG). 50% (12/24) were pts with secondary AML, including patients with secondary AML, demonstrates a high remission rate with acceptable side effect profile resulting in low induction mortality.

Summary/Conclusions: The addition of a novel E-selectin antagonist, GMI-1271, to anthracycline-based induction chemotherapy in untreated elderly pts with AML, including patients with secondary AML, demonstrates a high remission rate with acceptable side effect profile resulting in low induction mortality. This study compares favorably to previous studies (Lancet, ASCO 2016). E-selectin ligand was expressed on leukemic blasts in the majority of pts, therefore supporting its relevance as a target. A randomized trial is being planned.

P205
CM942 IS A NEW SMALL MOLECULE THAT TARGETS SET-PP2A INISTRIBUTION AND INHIBITS GROWTH OF ACUTE MYELOID LEUKEMIA CELLS
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Background: Acute myeloid leukemia (AML) is a heterogeneous malignant disorder of hematopoietic progenitor cells in which several genetic and epigenetic aberrations have been described. Nevertheless, outcome for most patients is poor, and it is necessary to develop more effective treatment strategies.

Aims: To test the efficacy of CM942, a FTY720 analogue, on AML cell lines and primary patient samples, and investigate its mechanism of action.

Methods: AML cell lines and 29 de novo AML samples were analyzed by treatment with FTY720 and CM942, MTS (viability), apoptosis, cell cycle and PDGFA activity assays, and western blot.

Results: CM942 exhibited notable cytotoxicity on all human AML cell lines with SET overexpression (n=10). Using phosphatase assays we confirmed that CM942 treatment activated PP2A on cell lines, similarly to FTY720. Immunoprecipitation of PP2Ac in untreated cells confirmed that SET interacts with PP2A, and that treatment with CM942 effectively disrupted this association. Furthermore, CM942 had a caspase-dependent pro-apoptotic effect, and decreased phosphorilation of the PP2A partner ERK1/2. Microarray data from vehicle-treated and CM942-treated HL-60 cells showed a high correlation between the gene expression profiles of the samples. This analysis identified up-regulated and down-regulated genetic pathways by treatment with CM942, providing mechanistic insights into the anti-tumor mechanism of this small molecule. Our analyses in primary AML samples showed that 7 out of 29 (24%) samples treated with CM942 had a significant reduction in proliferation.

Summary/Conclusions: CM942 inhibits growth of AML cells in both cell lines and primary patient samples, exerting its antileukemic effects through reactivation of PP2A activity. Although treatment with FTY720 was somewhat more effective than CM942 in primary samples of AML, fewer cytotoxic effects were observed after CM942 treatment in peripheral blood from healthy donors. Further experiments would be necessary to confirm the in vivo anti-tumor activity of CM942 in AML models. New compounds have been developed for the treatment of AML, although few have been translated into clinical practice; nevertheless, it is unlikely that any of these compounds, when used alone, will cure the disease, or decrease the relapse rate in poor comorbid conditions. The combination of glasdegib with ICT was well tolerated, with a safety profile consistent with that in AML pts receiving standard ICT. Further studies are warranted.

Table 1. mOS in Pts >60 yrs Stratified by European Leukemia Net (ELN) Risk Criteria

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>ICT (Historical Rolllig et al, 2011) months</th>
<th>ICT + Gladselig (n=44) months</th>
<th>Increase in mOS (%) (20 events)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
<td>14.6</td>
<td>Not reached (n=99)</td>
<td>Not estimable</td>
</tr>
<tr>
<td>Int-1</td>
<td>15.7</td>
<td>(n=12)</td>
<td>65.3</td>
</tr>
<tr>
<td>Int-2</td>
<td>14.3</td>
<td>(n=12)</td>
<td>45.7</td>
</tr>
<tr>
<td>Adverse</td>
<td>8.5</td>
<td>(n=10)</td>
<td>77.1</td>
</tr>
</tbody>
</table>

*1 pt was not classifiable by ELN risk.

Summary/Conclusions: Although the CR rates do not appear to be higher than those reported historically for AML pts receiving ICT, the mOS for AML pts >60 yrs stratified by subgroup compares favorably by adding glasdegib. This study demonstrates that this is the first trial of the use of glasdegib on the leukemia stem cells. The combination of glasdegib with ICT was well tolerated, with a safety profile consistent with that in AML pts receiving standard ICT. Further studies are warranted.
CLONAL HETEROGENEITY IN LEUKEMIC STEM CELLS FROM PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Clonal heterogeneity occurs in many cancers, including Acute Myeloid Leukemia (AML). In cases of remission, chemotherapy has triggered clonal selection with minor or evolved sub-clones driving relapse. A better understanding of the underlying clonal architecture, the extent of genetic heterogeneity and its response to therapy is necessary to better understand mechanisms of therapy escape and relapse.

Aims: In this study we aim to define the clonal architecture of AML during the course of therapy and in leukemia propagating cells.

Methods: We sequenced 12 AML samples at the time of diagnosis and in one case also at the time of relapse with at least 80% blasts per sample. 6/12 patients displayed a normal karyotype while the other 6 patients showed various cytogenetic abnormalities (inversion 16 (2), trisomy 8 (1), add(19)(p13.3) (1), complex aberrant karyotype (2)). Whole-exome sequencing (WES) was performed with the appropriate germ line controls. WES data were clustered using empirical Bayesian clustering.

Results: WES identified more than 3000 variants in total. By setting distinct filtration criteria (20% allele frequency (AF), ≥10 reads coverage, ≥2 reads support of the detected variant, SIFT-score <0.05 and GMAF <5%) 64 leukemia mutations were detected by SureSelect targeted capture exon sequencing (Agilent) of 295 genes that are recurrently mutated in hematologic malignancies (median coverage 507x [range: 111-777x]). Longitudinal mutation analysis had a trend for poor CR (Table 1). The median follow up duration of the 19 patients whose longitudinal specimens were sequenced was 17.2 months, blast clearance was rapid (median 8 weeks), and maximum clinical benefit required prolonged therapy (>6 months) in some patients.

Aims: Our aim was to understand the impact of somatic mutations and their clearance on disease response and survival outcomes in AML patients treated with pracinostat+AZA.

Methods: 88 samples from 41 study patients were sequenced. Pre-treatment samples were available for analysis from 41 patients, and a median of 3 longitudinal samples were analyzed from patients between Cycle 2 and 9. Leukemia mutations were detected by SureSelect targeted capture exon sequencing (Agilent) of 295 genes that are recurrently mutated in hematologic malignancies (median coverage 507x [range: 111-777x]). Longitudinal mutation analysis was characterized by tracking variant allele frequency (VAF). Informed consent was obtained from all patients.

Results: At baseline, 96 mutations in 28 genes were detected in 38 (93%) patients, with the most frequent being in SRSF2 (27%), DNMT3A (20%), IDH2 (17%), RUNX1 (17%), and TET2 (17%). The median number of mutations detected per patient was 2 (range: 0-6). Among the 33 patients with evaluable treatment response, CR was observed in 13 (39%) patients. The rate of CR was significantly higher in patients with mutations in genes associated with DNA methylation, RNA splicing, clonal hematopoiesis of indeterminate potential (CHIP), and receptor tyrosine kinase (RTK) pathways had poor clearance of mutation, while transcription factors or cohesin had better clearance with pracinostat+AZA treatment. In 2 patients, relapsed samples were sequenced and showed re-expansion of the founder clone.

Table 1.

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Summary/Conclusions: WES can identify leukemia specific mutations that are involved in various cellular functions including mutations that have been shown to be recurrently mutated in AML like DNMT3A. Sequencing data can also be used in combination with mathematical modelling approaches to reconstruct the clonal architecture of AML at the time of diagnosis and relapse allowing estimations of the clonal complexity at these time points.
Summary/Conclusions: Mutations in NPM1, and DNA methylation pathway were associated with a better response to pracinostat+AZA, while TP53 mutation was associated with a trend toward poor response. Persistent mutation at the time of CR suggests residual preleukemic clonal hematopoiesis in this elderly population. Benefit of prolonged exposure to pracinostat+AZA was also confirmed at molecular level where continued decline of mutation VAF was seen after achieving CR.

Acute myeloid leukemia - Clinical 3

P208

STABLE DISEASE WITH HEMATOLOGIC IMPROVEMENT IS CLINICALLY MEANINGFUL FOR OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA TREATED WITH AZACITIDINE

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Background: Effects on overall survival (OS) are of primary importance when evaluating AML treatments (Tx). Though complete remission (CR) rates are lower with azacitidine (AZA) than with intensive chemotherapy (IC), OS is similar with AZA and IC (Dombret et al., Blood, 2015). The 2017 European LeukemiaNet (ELN) recommendations acknowledge that hypomethylating agents, including AZA, may alter the natural course of AML in some patients (pts) who do not achieve CR (Döhner et al., Blood, 2017). According to IWG criteria for AML (Cheson et al., J Clin Oncol, 2003), stable disease (SD) is considered non-response to Tx. Yet AML is a progressive disease; potentially, stable health status may reflect delayed disease progression and result in improved OS.

Aims: This post hoc analysis evaluated OS outcomes among older pts with AML treated with AZA or conventional care regimens (CCR) who maintained SD, with or without hematologic improvement (HI), in the phase 3 AZA-AML-001 study.

Methods: Pts aged ≥65 years with AML (>30% marrow blasts), ECOG PS score ≤2, NCCN-defined intermediate- or poor-risk cytogenetics, and WBC count ≤15x10⁹/L received AZA (75mg/m²x7 days [d]/28d cycle) or a CCR (IC [standard 7+3 regimen], low-dose cytarabine [20mg BID x 10d/28d cycle], or best supportive care). OS was assessed using Kaplan-Meier methods for pts with SD at 2-, 4-, and 6-month landmarks. SD was protocol-defined as the absence of an IWG-defined AML response and no progressive disease (PD), whether or not HI was attained. Pts with SD could have had an IWG-defined response or PD at any time other than at the specified landmarks. OS was also evaluated in pts with HI as their best response; attainment of HI must have begun on or before, and been sustained past, each landmark, and lasted for ≥56 consecutive days.

Table 1.

Results: Median OS for all SD pts was 2.1-2.5 months longer with AZA vs CCR, and estimated 1-year survival was ~15% higher at each landmark in the AZA arm (Table 1). Hazard ratios for OS among all SD pts treated with AZA vs CCR ranged from 0.81–0.88. Median OS among pts with SD and no HI ranged from 12.6–13.3 months in the AZA arm and from 11.1-12.2 months in the CCR arm. Within Tx arms, AZA-treated pts with HI had meaningfully improved OS at all landmarks, ranging from 3.7 to 7.9 months longer than OS for pts without HI (Table 1). In contrast, HI attained with CCR did not largely influence OS; differences between pts who attained HI vs no HI ranged from -0.2 to 2.9 months. Median durations of HI in the AZA vs CCR arms, respectively, were 183 vs 166
days at 2 months, 176 vs 148 days at 4 months, and 176 vs 138 days at 6 months. Estimated 1-year survival within the AZA arm was 4.9%–27.4% greater for pts with HI than for pts with no HI, but for CCR-treated pts with HI, 1-year survival was 0%–10.3% greater. Between Tx arms, 1-year survival with AZA in pts with HI was 9.6%–33.3% greater than for CCR-treated pts with HI.

Summary/Conclusions: Maintaining SD during AZA or CCR Tx is associated with relatively favorable OS outcomes, as median OS in pts with SD exceeded that for all pts in the AZA-AML-001 trial (10.4 months with AZA vs 6.5 months with CCR; Dombret et al., Blood, 2015). Pts with SD who also attained HI during early AZA Tx had meaningfully improved OS, whereas similar CCR-treated pts did not, suggesting that HI with AZA is qualitatively different from HI with CCR. The prognostic relevance of HI in AML requires further study.

P209

A RANDOMIZED PHASE II STUDY OF IDARUBICIN AND CYTARABINE WITH EITHER CLOFARABINE OR FLUDARABINE IN ADULTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA WITH EITHER CLOFARABINE OR FLUDARABINE IN ADULTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA (AML).

Aims: We designed a randomized phase II trial to evaluate the efficacy and safety of idarubicin and cytarabine with either clofarabine (CIA) or fludarabine (FIA) in adults with newly diagnosed AML. The primary objective was to compare the EFS rates of the two regimens.

Methods: Adults with newly diagnosed AML deemed suitable for intensive chemotherapy were randomized using a Bayesian adaptive design to receive CIA or FIA. All patients (pts) received idarubicin 10 mg/m² IV on D1-3 and cytarabine 1 g/m² IV daily on D1-5. Clofarabine and fludarabine were given at doses of 15 mg/m² and 30 mg/m², respectively, IV daily on D1-5. Pts with ITD mutations could receive concomitant sorafenib. Responding pts could proceed to a historical cohort of pts <60 years of age who received idarubicin and cytarabine (IA) without a nucleoside analogue.

Results: Between 8/2011 and 6/2016, 182 pts were enrolled (CIA, n=106; FIA, n=76; Table 1).

Table 1.

The imbalance of the arms was due to the better performance of CIA during the initial period of the trial. Treatment arms were well-balanced after randomization. 12 pts (55%) in the CIA arm and 8 (33%) in the FIA arm received sorafenib. The composite CR/CRp rate was similar between the two arms (80% for CIA vs 82% for FIA; P=0.84). CR was achieved in 72% and 74% in the CIA and FIA arms, respectively. MRD negativity rates at remission by multiparametric flow cytometry were higher in the CIA arm (80% vs 65%; P=0.07). 37 pts (35%) in the CIA arm and 28 (38%) in the FIA arm underwent allogeneic stem cell transplant in first remission. The median duration of follow-up was 27 months (range, 1-58). Median EFS for pts who received CIA and FIA were 13 months and 17 months, respectively; the 2-year EFS rate was 44% in both arms (P=0.91). Median OS were 24 months and not reached, and the 2-year OS rates were 51% and 57%, respectively (P=0.23). No differences in EFS or OS were observed according to baseline factors, including cytogenetics, mutations or ELN risk group. CIA was generally associated with more adverse events compared to FIA, including a higher rate of transaminase elevation (29% vs 4%), hyperbilirubinemia (26% vs 9%), and rash (29% vs 12%). Early mortality was similar in the 2 arms (60-day mortality: 4% for CIA vs 1% for FIA; P=0.32). We compared outcomes of pts treated with either CIA/FIA to a historical cohort treated with IA (n=92). Pts in the CIA/FIA group with FLT3 mutations who received sorafenib (n=20) were excluded from this analysis. The two arms were similar with respect to pretreatment characteristics analyzed, including age, cytogenetics, and ELN risk. No differences were observed in CR/CRp rates, EFS or OS between the two groups. However, among pts <50 years of age, the median EFS for pts who received FIA (n=36), CIA (n=28) and IA (n=34) was not reached, 10 months and 9 months, and the 2-year EFS rates were 58%, 33% and 30%, respectively (P=0.05 for FIA vs IA; P=0.79 for CIA vs IA). For these pts <50 years of age, the median OS was not reached, 22 months and 15 months, and the 2-year OS rates were 72%, 46% and 36%, respectively (P=0.009 for FIA vs IA; P=0.23 for CIA vs IA).

Summary/Conclusions: We have observed similar efficacy in younger pts with newly diagnosed AML, although FIA is associated with a better toxicity profile. FIA may improve outcomes compared to IA in pts <50 years of age.

P210

OVERALL SURVIVAL AND TRANSPLANTATION IN PATIENTS WITH FLT3 MUTATIONS: SUBGROUP ANALYSIS OF A PHASE 3 STUDY OF CPX-351 VERSUS 7+3 IN OLDER ADULTS WITH NEWLY DIAGNOSED, HIGH-RISK ACUTE MYELOID LEUKEMIA


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Background: Fludarabine and clofarabine are purine nucleoside analogues that deliver a synergistic 5:1 molar ratio of cytarabine and daunorubicin. CPX-351 demonstrated significantly prolonged overall survival (OS) versus cytarabine/daunorubicin (7+3) in a randomized, open-label, controlled phase 3 trial in patients aged 60 to 75 years with newly diagnosed, high-risk AML (Lancet, et al ASCO 2016). A study of the ex vivo cytotoxicity of CPX-351 found that AML blasts with the FLT3-ITD phenotype were 5-fold more sensitive to CPX-351 than those with wild type FLT3 (Gordon, et al Leuk Res. 2017;53:39-49).

Aims: The current analysis of the phase 3 trial therefore investigated outcomes in the subset of patients with FLT3 mutations.

Methods: Enrolled patients were randomized 1:1 to receive induction with 1 to 2 cycles of CPX-351 (100 units/m² [cytarabine 100 mg/m²+daunorubicin 44 mg/m²] on Days 1, 3, and 5 [2nd induction: Days 1 and 3 only]) or 7+3 (cytarabine 100 mg/m²/day x 7 days [2nd induction: x 5 days]+daunorubicin 60 mg/m² on Days 1, 2, and 3 [2nd induction: Days 1 and 2 only]). Patients who achieved complete remission (CR) or CR with incomplete platelet or neutrophil recovery (CRi) could receive up to 2 consolidation cycles.

Results: Of the 274 patients who were assessed for FLT3 mutations and received studied treatment, 22/138 (16%) patients in the CPX-351 arm and 20/136 (15%) patients in the 7+3 arm had baseline FLT3 mutations. AML subtypes in FLT3+ patients were: therapy-related AML (19%); AML after myelodysplastic syndrome (MDS) for which otherwise myelodysplasia was a poor-risk AML subtype (11%); and de novo AML with MDS karyotype (21%). In FLT3+ patients, median OS was longer with CPX-351 (10.25 months) versus 7+3 (4.55 months; hazard ratio=0.57 [95% CI: 0.24, 1.33]; P=0.093; see Figure 1), and the rate of CR+CRi was higher (68% vs 25%). A greater number of FLT3+ patients treated with CPX-351 were able to undergo stem cell transplantation (n=10/22 [45%]; 4 patients were alive as of this analysis, after a median post-transplant follow up of 692 days [range: 96-769]) compared with 7+3 (n=22/101 [10%]; neither patient was still alive). The adverse event profile (reported during treatment or within 30 days of discontinuation) in CPX-351 in FLT3+ patients was consistent with the overall study population. Serious treatment-emergent adverse events (TEAEs) were experienced by 7 (32%) FLT3+ patients in the CPX-351 arm and 10 (50%) patients in the 7+3 arm; individual serious TEAEs in ≥2 patients included febrile neutropenia (n=2 in each arm), respiratory failure (n=1 and 2), and cardiac failure (n=2 with CPX-351), and cerebral hemorrhage (n=2 with 7+3).

Summary/Conclusions: CPX-351 demonstrated numerical improvement in median OS in older patients with newly diagnosed, FLT3+ high-risk AML and
allowed more patients to undergo stem cell transplantation. The safety of CPX-351 in this subpopulation was in line with the previous studies and the overall phase 3 population. This analysis was limited by small number of patients.

### P211

**NIVOLUMAB MAINTENANCE THERAPY FOR PATIENTS WITH HIGH-RISK ACUTE MYELOID LEUKEMIA IN REMISSION**

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**Background:** Dose intensification and newer drug combinations during induction have led to high rates of complete remission (CR) in pts with newly diagnosed AML. However, disease relapse remains a major source of failure. With the exception of allogeneic (allo) stem cell transplant (SCT), there are few options for post-consolidation maintenance of remission in high-risk pts. Prior attempts at maintenance therapy with 1 of toxic drugs have been unsuccessful. Immune mediated disease control by engaging tumor-specific cytotoxic T-cells may be important in suppressing leukemia relapse, as is seen with graft vs leukemia effect following allo SCT. Immune checkpoint inhibitors may be effective in restoring host immune surveillance in the setting of post-remission therapy.

**Aims:** We designed a pilot phase II clinical trial studying the efficacy and safety of nivolumab (nivo) as maintenance therapy in AML pts with high-risk disease in remission, who were not being considered for SCT.

**Methods:** AML pts ≥18 years with a high-risk feature in 1st CR (CR1) or any patient in 2nd CR (CR2) who had received induction and at least 1 consolidation cycle were eligible for enrollment. Pts should be within 12 months of achieving CR, have PS ≤2, and adequate organ function. Pts were treated with nivo 3mg/kg IV every 2 weeks for 6 months. 1 cycle was 4 weeks. After 6 months, nivo could be given every 4 weeks until 12 months on study, and then every 3 months until relapse. All pts had baseline cytogenetic and molecular testing, and minimal residual disease (MRD) assessment by flow cytometry. Peripheral blood and bone marrow samples were collected at baseline and during treatment for immune correlative studies to explore immune cell repertoire and biomarkers for response.

**Results:** Eight pts have been treated, with a median age of 60 years (range, 49-71). 7 pts were in CR and 1 in CRi at the time of enrollment; 5 pts (63%) were in CR1, 2 pts (25%) were in CR2, and 1 pt (13%) in CR4 was inadvertently enrolled. Pts were treated with nivo 3mg/kg IV every 2 weeks for 6 months. 1 cycle was 4 weeks. After 6 months, nivo could be given every 4 weeks until 12 months on study, and then every 3 months until relapse. All pts had baseline cytogenetic and molecular testing, and minimal residual disease (MRD) assessment by flow cytometry. Peripheral blood and bone marrow samples were collected at baseline and during treatment for immune correlative studies to explore immune cell repertoire and biomarkers for response.

**Summary/Conclusions:** Nivo appears to be a feasible maintenance strategy in high-risk AML pts who are not candidates for SCT. The study continues to surpass the pre-specified expected rate of 6-month relapse-free survival of high-risk pts based on a historical cohort. Correlative studies profiling the immune repertoire of pts before and during treatment are being finalized and will be summarized.

### P212

**HIGHER EXPRESSION OF LONG NON-CODING RNA KIAA0125 IS ASSOCIATED WITH CHARACTERISTIC CLINICAL AND BIOLOGICAL FEATURES AND IS AN INDEPENDENT POOR PROGNOSTIC FACTOR IN ACUTE MYELOID LEUKEMIA**

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**Background:** Long non-coding RNAs (IncRNAs) are non-protein coding RNAs longer than 200 nucleotides. Recently, a number of IncRNAs have been shown to play important roles in cancer biology. IncRNA KIAA0125 is one of the 11 genes in an expression signature significantly associated with prognosis in cytogenetically normal acute myeloid leukemia (AML) patients as shown in our previous report. It is also among another set of 17 leukemia stem cell (LSC) genes, identified through xenotransplantation model in NSG mice, which predict inferior treatment response in AML.

**Aims:** KIAA0125 gene is localized on chromosome 14q32.33; its functions remain unexplored. One study reported that it might be involved in neurogenesis including induction of astrocytosis, preventing formation of dopaminergic neurons. Another study showed that it could potentiate cell invasion and migration in gallbladder cancer. Its clinical significance in hematologic malignancies has not been explored yet. Since independent studies have reported KIAA0125 as an important gene for unfavorable prognosis, in this study we aimed to investigate its clinical relevance in AML.

**Methods:** We performed global mRNA arrays for bone marrow samples from 347 newly diagnosed de novo AML patients in the National Taiwan University Hospital, who had adequate cryopreserved cells and detailed demographic, clinical, and genetic data for analysis. The KIAA0125 expression level extracted from the array data was analyzed for its clinical relevance. We also validated our findings by analyzing the public databases of AML.

**Results:** The 347 patients were divided into two groups based on the median level of KIAA0125 expression on the arrays. Higher KIAA0125 expression was inversely associated with favorable karyotypes including t(8;21) and t(15;17). Patients with M1 by the French-American-British classification more frequently had higher KIAA0125 expression (p < 0.001), while those with M3 (acute promyelocytic leukemia) had significantly lower levels of KIAA0125 expression (p < 0.001). To investigate the association of gene mutations with KIAA0125 expression in AML, we analyzed mutations of 17 AML-associated genes. We found that patients with higher KIAA0125 expression had significantly higher incidence of FLT3-ITD (28.7% vs 19.7%, p = 0.048), and mutations of RUNX1 (18.4% vs 10.4%, p = 0.034), and DNMT3A (24.1% vs 13.9%, p = 0.015), compared to those with lower KIAA0125 expression. Among the 227 patients who received standard chemotherapy, those with higher KIAA0125 expression had a lower complete remission rate (61.2% vs 84.7%, p < 0.001), and shorter overall survival (median OS, 23.7 months vs 116.8 months, p = 0.001) than those with lower KIAA0125 expression after a median follow-up of 57.0 months. The prognostic significance could be validated in another two independent cohorts, TCGA and GSE12417. In multivariate analyses, higher expression of KIAA0125 remained to be an unfavorable prognostic factor for OS independent of age, white blood cell counts, karyotype, FLT3-ITD, CEBPA double mutations.
RUNX1 mutation, MLL-PTD, WT1 mutation, and TP53 mutation (p=0.011).

Summary/Conclusions: Higher expression of KIAA0125 in AML patients was correlated with mutations of RUNX1, DNMT3A, and FLT3-ITD but negatively associated with favorable karyotypes such as t(8;21) and t(15;17). Higher expression of KIAA0125 appeared to be an independent unfavorable prognostic factor in our cohort, and its negative prognostic impact could be validated in another two large independent cohorts of AML. The close association of KIAA0125 expression with LSC signatures might in part explain its unfavorable impact on the survival of AML patients.

P213

LEUKEMIC STEM CELLS CAN BE DETECTED IN A CONSIDERABLE PERCENTAGE OF PATIENTS WITH ACUTE MYELOID LEUKEMIA AT DIAGNOSIS AND IS A SIGNIFICANT PROGNOSTIC FACTOR

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Background: There is a growing interest on the identification of leukemic stem cells (SC) as a potential prognostic factor in patients with acute myeloid leukemia (AML). Several studies identify these cells as CD34+CD38-Lin-, although there is a controversy about its phenotypic identification and prognostic value.

Aims: To identify SC in a cohort of patients with AML and evaluate their prognostic value in a series of newly diagnosed AML patients.

Methods: The presence of SC (CD34+CD38-Lin-) in bone marrow samples was prospectively evaluated in a consecutive series of 67 newly diagnosed AML patients by flow cytometry, between may 13-Oct. 16. All patients received intensive chemotherapy according to PETHEMA protocol. We evaluated response, relapse rate and overall survival (OS) and event free survival (EFS).

Results: Out of the 67 patients [34 men/33 women, median age 54 (0-78)], 58 (88.6%) have SC at diagnosis, 37.9% of them (n=22) achieved complete remission (CR) with a negative minimal residual disease (MRD) vs 77.8% (7/9) among patients without SC (p=0.03). Among patients who obtained CR with a negative MRD (n=29), no one suffer a leukemic relapse in the non SC vs 5/22 (22.7%) in the SC group (p=0.02). Considering the intermediate risk group according to cytogenetic / molecular features, 100% of patients without SC at diagnosis achieve a negative MRD (5/5) vs 14/41 (34.1%) among those in the SC group (p<0.008). OS at 9 months was 89 vs 56% (p=0.043), and the EFS 78 vs 48% (p=0.054) in the non SC and SC group, respectively (Figure 1).

Figure 1.

Summary/Conclusions: SC can be detected in a considerable group of patients with AML at diagnosis. The presence of SC is a prognostic factor in terms of response, OS and EFS. Accordingly, SC detection could help to identify prognosis subgroup of patients with different prognostic among those in the intermediate risk group by genetics/molecular assays.

P214

POST-REMISSIONAL AND PRE-TRANSPLANT ROLE OF MINIMAL RESIDUAL DISEASE DETECTED BY WT1 IN ACUTE MYELOID LEUKEMIA: A RETROSPECTIVE COHORT STUDY

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Background: In acute myeloid leukemia (AML) the detection of residual leukemic cells at a submicroscopic level (minimal residual disease - MRD) is still under investigation. In about 30-40% of AML lacking a specific molecular target, quantitative real-time polymerase chain reaction (QRT-PCR) has been used to detect transcripts commonly overexpressed in AML. Among a large number of candidates, Wilms tumor gene 1 (WT1) has been proposed as a promising MRD marker.

After the standardization of QRT-PCR on behalf of the European LeukemiaNet (ELN), subsequent studies investigated the role of WT1 expression in AML with controversial results.

Aims: To assess the role of WT1 expression as a MRD marker after intensive induction chemotherapy and before allogeneic hematopoietic cell transplantation (allo-HCT) in a large cohort of AML patients treated in a single institution.

Methods: The present retrospective cohort study included adult patients with untreated AML consecutively diagnosed between 2004 and 2014 in the Hematology Unit of the University-Hospital Città della Salute e della Scienza of Torino, Italy. The study was approved by the Ethical Committee and was registered at www.clinicaltrials.gov as NCT02714790. Among 255 enrolled patients, MRD was investigated in those in first complete remission (CR) with an available at diagnosis and at two further time-points: after induction (n=117) and prior allo-HCT (n=65).

Results: Baseline WT1 expression lacked even to show an association with response to induction chemotherapy (OR 1.16; 95% CI 0.90-1.50, p=0.24).

Conclusions: OS and DFS were significantly shorter in patients in first CR with >350 WT1 copies after induction compared to those with ≤350 (OS 17 vs 9 months with HR 2.13; 95% CI 1.14-3.97, p=0.018 and 3-year DFS rates 15% vs 55% with a HR of 2.81; 95% CI 1.14-6.93, p=0.025).

Adding the BM WT1 in the model along with other factors determines an increase of the C-statistic from 0.696 to 0.713 for OS (NRI=0.384) and from 0.7413 to 0.7920 (NR=0.4037) for DFS. Before allo-HCT, patients with WT1 >150 copies (n=18) had a significantly higher CIR compared to those with WT1 ≤150 (n=47), HR 4.61; 95% CI 1.72-12.31, p=0.002.

Summary/Conclusions: The results of the present study showed that BM WT1 is associated with survival in patients in CR in two decisive time-point for treatment planning: after induction treatment and before allo-HCT. The prognostic role of WT1 resulted independent from other well-established risk factors. Therefore, WT1 may represent an additional MRD tool for risk stratification in patients nowadays classified in CR, especially in the high risk MRD positive subgroup in which a risk-adapted approach may have a role. Published evidences available so far supported these suggestions, but mainly due to methodological issues, the role of WT1 is still a matter of debate. Perspective randomized studies are required to confirm these results.

P215

DIFFERENTIATION SYNDROME ASSOCIATED WITH ENASIDENIB (AG-221), A SELECTIVE INHIBITOR OF MUTANT ISOCITRATE DEHYDROGENASE 2 (MIDH2)

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Background: Enasidenib (AG-221) is an oral, selective, small-molecule inhibitor of miHDH2 enzymes. Preclinical studies showed that exposing myeloblasts from patients (pts) with acute myeloid leukemia (AML) to enasidenib ex vivo resulted in differentiation of leukemic marrow blasts into mature, functional neutrophils (Cancer Discov, 2017). Vian et al. showed that enasidenib may result in IDH-inhibitor-associated differentiation syndrome (IDH-DS) in treated pts, with manifestations akin to retinoic acid syndrome seen during therapy of acute promyelocytic leukemia.

Aims: To characterize the prevalence, characteristics, and course of IDH-DS in enasidenib-refractory or refractory (R/R) AML receiving enasidenib 100 mg daily in a phase 1 dose-escalation and expansion study (NCT01915498). This dose is currently under study in a multicenter, randomized, phase 3 trial comparing enasidenib with conventional care regimens in R/R AML pts (NCT02577406).
Aggressive Non-Hodgkin lymphoma - 1st line

P216
Abstract withdrawn.

P217
OUTCOME OF PATIENTS WITH INTRAVASCULAR B-CELL LYMPHOMA, A RETROSPECTIVE STUDY CONDUCTED ON BEHALF OF THE LYMPHOMA STUDY ASSOCIATION GROUP


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Background: Intravascular large B-cell lymphoma (IVLBCL) is a rare type of extranodal large B-cell lymphoma characterized by the selective growth of lymphoma cells within the lumina of vessels, classically reported with poor responses to chemotherapy. Due to its low incidence and rarity of tumor cells, diagnosis of IVLBCL remains difficult and many issues remain unresolved, regarding both clinical features and therapeutic strategies.

Aims: Our work aims to describe clinical presentation and outcome of IVLBCL patients treated in French LYSA centers between 2000 and 2016. All LYSA centers were asked to report and update clinical data about IVLBCL patients treated. No central pathology review was performed for the present study, but all cases were classified by LYSA pathologists. Local investigators reported disease characteristics and updated patients’ outcome (clinical examination, standard biological parameters, bone marrow biopsy, CT scan at baseline, CT response evaluation and outcome).

Results: We identify 65 IVLBCL patients treated in 23 LYSA centers during the studied period. Median age was 67.8 years (range 22-91). In note, two third of patients presented with IPI score >3 (67%) and all patients had a stage IV disease. As expected in Western patients, cutaneous and CNS involvement were highly frequent, respectively 33% and 39%. But interestingly, hemophagocytic syndrome were observed in nearly half of the patients (41%), while it was mainly described in Asian series. Despite classically delayed diagnosis in IVL-BCL, only 2 cases were confirmed post-mortem and almost all alive patients at diagnosis (n=58) were treated with rituximab-containing chemotheraphy regimen (92%). Regarding first line treatment, 83% of patients were treated with anthracycline-based regimens, with CNS prophylaxis for half of them (47%), and seven patients underwent autologous stem cell transplantation upfront. The median progression free survival was 28.4 months and median overall survival was 63.8 months (Figure 1). Pathological features (including cell of origin characterization, C-MYC expression, adhesion protein expression level) investigation is ongoing and will be presented at the time of the meeting.

Figure 1.
P218
OUTCOME OF ELDERLY DLBCL PATIENTS (≥80 YEARS) TREATED WITH ANTHRACYCLINE BASED CHEMOTHERAPY; R-CHOP DOSE REDUCTION IS NOT NECESSARY FOR EVERYBODY

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Summary/Conclusions: The present study is the largest European IVLBCL series. It shows that despite the use of modern immune-chemotherapy, IVLBCL remains an aggressive lymphoma entity. In particular, these patients are highly exposed to early relapse and therefore should be considered for innovative frontline therapies.

Background: Management of elderly patients (above ≥80y) is difficult and only limited number of patients could be treated by curative approach with anthracycline based chemotherapy. Dose reduction of particular drugs is used very often and it varies based on pt's characteristics and center preferences. There is however lack of randomized or at least non-randomized historical comparisons.

Aims: The objective of this study is to analyze elderly DLBCL patients prospectively registered in NIH Lymphoma Project and treated anthracycline based regimen in real world outside of clinical trials.

Methods: Patients (pts.) with informed consent are prospectively followed in multicenter Lymphoma Project since 1999. Diagnostic, therapeutic and follow up data are prospectively collected. There were 399 DLBCL pts older than 80year diagnosed in period 1999-2014 identified. Among 372 pts. with pathology review and essential data there were 112 pts. (30.1%) treated with R-CHOPlike chemotherapy. Analysis of clinical prognostic factors, therapy and toxicity was performed. Pearson, Kaplan-Meier and log rank tests were used.

Results: Median age was 81 years (80-88), 51.8% of men. Proportion of pts. ≥85 was 14.3%, with PS ≥2 (ECOG) 34.0%, with higher LDH 64.3%, with BMI or intermediate high IPI 49.1%, with bulky disease (≥10 cm) 17.0%, with visceral disease (≥50%) 17.7%, with Charlson Comorbidity Score (CCS) ≥4 25%, Charlson Comorbidity Score (CCS) ≥4 25%. According to treatment choice of physician (intent to treat), pts. could be divided into 3 groups R-CHOP (CH) (cyclophosphamide -CF 750 mg/m2, adriamycin – A - 50 mg/m2) or R-MiniCHOP (minich) (CF 400 mg/ m2, A 25 mg/m2, Peryade 2011) or modified R-CHOP (modich) (CF 750 mg/m2 and A 25 mg/m2 or any other dose between CHOP and miniCHOP). There were 21 pts (15.8%) treated with CH, 38 (33.9%) with miniCH and 53 (47.3%) with modified CH. There were no significant differences between the subgroups, except higher proportion of bulk in modich vs miniCH and CH (35% vs 12.9% vs 7.7% resp; p 0.04) and cardiac comorbidities (60.5% vs 33.3% vs 30.2% resp.; p 0.02). Six and more cycles were administered in 71.4%, 63.1% and 58.5% pts. in CH, miniCH and modich resp. Following proportion of pts. received >80 (>50%) of original CHOP dose. For cyclophosphamide it was 66.7% (81%), 0% (50%) and 62.2% (79.2%) resp. and for A it was 57.1% (76.1%), 2.6% (15.8%) and 13.2% (49%) resp. for CH, miniCH and modich resp. There were observed 11 treatment related deaths (6 cardiac toxicity and infection), S in miniCH and 6 in modich groups. The overall response rate was 76.8% with 59.8% CR/CRu. Median PFS and OS were 2.8y and 3.5y resp. (Figure 1A) with median follow up of 3.3y. There were found high beta2microglobulin (HR 2.2, p 0.05), low albumin (HR 1.9, p 0.05) and PS (p 0.05) as the only factors correlated with OS as well as PFS (data not shown). Pts who achieved CR or PR have significantly better OS (median (as shown). Pts who achieved CR or PR have significantly better OS (median (as well as PFS) compared to stable or progressive disease with 4.6 vs 3.5 vs 0.8 vs 0.5 y. There was numerically (not significantly) better OS median for R-CHOP (4.6y) vs R-miniCHOP (3.2y) and R-modichOP (2.9y) (Figure 1B).

Figure 1. Summary/Conclusions: Only one third of elderly DLBCL pts (≥80y) is treated with anthracycline based regimen. Performance status, albumin and beta2microglobulin levels were significantly associated with prognosis. In minority of these pts full dose of R-CHOP could be safely used and there is trend to better overall survival.

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P219
IMPROVED SURVIVAL IN PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA UP TO AGE 70 ONLY: A POPULATION-BASED STUDY ON INCIDENCE, PRIMARY TREATMENT AND SURVIVAL IN THE NETHERLANDS, 1989-2015

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Background: PCNSL is a rare, aggressive form of an extranodal non-Hodgkin lymphoma that exclusively affects the CNS. Recent findings from the few available prospective studies demonstrated improved outcome in PCNSL. However, the results from such studies are inherent to patient selection. Population-based studies that assess long-term patterns of incidence, treatment and survival in PCNSL are virtually lacking.

Aims: The aim of this comprehensive nationwide population-based study was to assess trends in incidence, primary treatment and survival among adult PCNSL patients (pts) diagnosed during a 27-year period in the Netherlands.

Methods: We selected all adult (≥18 years) pts diagnosed with PCNSL of the diffuse large B-cell type in the Netherlands between 1989-2015 from the nationwide Netherlands Cancer Registry with survival follow-up through February, 2016. Pts diagnosed without pathological or cytological confirmation (n=50) and pts diagnosed at autopsy were excluded (n=32). Age-standardized incidence rates (ASR) were calculated per 1,000,000 person-years and standardized according to the European standard population. Data on primary treatment (i.e. no therapy, chemotherapy (CT) alone, radiotherapy (RT) alone, and CT+RT) were available for individual pts. Pts were categorized into 4 periods (1989-1994, 1995-2000, 2001-2007 and 2008-2014) and 3 age groups (18-60, 61-70 and >70 years). We calculated relative survival (RS) and the relative excess risk of mortality as measures of disease-specific survival.

Results: We included a total of 1,673 newly diagnosed PCNSL pts in the study (median age, 65 years; age range, 19-89 years; 53% males). The ASR of PCNSL increased from 3.0 in the first period (1989-1996) to 4.4 in the last period (2009-2015), which was consistently higher among males than in females throughout the entire study (4.8 v 4.0 in the last period). The age-specific incidence rates were 2.3, 9.0 and 10.9 in the first period for the three age groups (18-60, 61-70 and >70 years), as compared with 2.7, 18.7 and 19.5 in the last period. The application of CT+RT increased exclusively among pts age 18-60. More specifically, the proportions for the three age groups were 26, 18 and 4% in the first period, as compared with 60, 10 and 4% in the last period. The use of CT alone among pts age >60 decreased with each period, following the wider use of CT alone over time, especially for pts age 61-70 years. The proportions of CT alone for the three age groups were 11, 8 and 2% in the first period, as compared with 31, 64 and 32% in the last period. Of note, 38 and 26% of pts age >70 received no therapy and RT alone in the last period, respectively. Five-year RS only improved for pts age 18-70 (Figure 1). Five-year RS (95% confidence intervals) was 22% (16%>30%), 13% (7%>22%), and 3% (1%>10%) in the first period for the three age groups, as compared with 56% (47%-64%), 35% (28%-43%) and 6% (2%-13%) in the last period. A multivariable survival model confirmed the adverse effect of older age on excess mortality and an improvement of survival over time. However, when information on treatment was added to that model, the effect of period lost statistical significance. This suggest that treatment contributed to the improved survival over time. Older age remained a predictor of poor prognosis.

Figure 1. Summary/Conclusions: The incidence of PCNSL steadily increases among

haematologica | 2017; 102(s2) | 57

Madrid, Spain, June 22 – 25, 2017
pts >60 years, which might in part be related to improved diagnostic practices among the elderly. Over time, RS increased over the past decades for pts 70 or below. This is largely explained by the increased use of intensive therapy over time. Although the use of CT alone gradually increased among pts >70 years, their survival is still poor. Therefore, there is an urgent need to design specific trials for elderly PCNSL pts to improve their survival.

P220

CLINICAL CHARACTERISTICS AND LONG-TERM RESULTS OF TREATMENT OF DIFFUSE LARGE HEPATITIS C-ASSOCIATED NON-HODGKIN LYMPHOMA (DLBCL+C)

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Background: In the WHO classification (2008), hepatitis C virus distinguishes as one of the etiological factors of multistage etiopathogenesis DLBCL. Aims: The purpose of this study was to evaluate clinical features and results of treatment of diffuse krepnokletochnoy lymphoma associated with hepatitis C in comparison with a control group of patients with diffuse large lymphoma without viral hepatitis markers.

Methods: It was included 521 patients with DLBCL: 98 patients with DLBCL and markers of hepatitis C (DLBCL+C) and a control group of 422 patients with DLBCL without markers of hepatitis C (DLBCL-C).

Results: Patient's age ranged from 21 to 76 years (median was 47 years) in DLBCL+C, ranged from 23 to 81 years (median 61) in DLBCL-C (p=0.02). The male:female ratio was 1: 1.3 in patients with DLBCL+C, 1: 1.7 in the group DLBCL-C. Stage I and II were in 11% patients with DLBCL+C, and 48% patients with DLBCL-C; III and stage IV were detected in 89% patients with DLBCL+C and 52% of DLBCL-C (p=0.00002). Extramedial lesions detected in 72% in DLBCL+C and in 26% in C DLBCL-C (p=0.006). In comparable groups localization of extramedial lesions was: spleen (52% to 23%), bone marrow involvement (43% and 27%), liver (42%) in DLBCL+C and 14% in DLBCL-C. Stage I and II were in 11% patients with DLBCL+C, and 48% patients with DLBCL-C; III and stage IV were detected in 89% patients with DLBCL+C and 52% of DLBCL-C (p=0.00002). Extramedial lesions detected in 72% in DLBCL+C and in 26% in C DLBCL-C (p=0.006). In comparable groups localization of extramedial lesions was: spleen (52% to 23%), bone marrow involvement (43% and 27%), liver (42%) in DLBCL+C and 14% in DLBCL-C.

Conclusion: The study is a prospective, clinical, single-centre study. The study aims to include 70 consecutive chemotherapy-naive lymphoma patients scheduled for intended curative chemotherapy without planned mediastinal radiation therapy. All patients undergo routine clinical examinations, but with supplementary imaging, including 1) baseline 82Rb PET and MR (prior to treatment); 2) acute 82Rb PET and MR (within 1 week of the first treatment); 3) subacute 123I-MIBG (after 2-3 months of therapy) and 4) late MR (1 year after the start of treatment). 82Rb PET imaging is performed at rest and during pharmacological stress testing with adenosine. It is primarily used to evaluate the acute effects of doxorubicin on myocardial perfusion. 123I MIBG is used for detection of doxorubicin-induced subacute changes in the myocardial adrenergic neurons. Cardiac MR is performed with late gadolinium enhancement and provides information on acute and late changes in left and right ventricular function, atrial and ventricular volumes, myocardial mass and interstitial fibrosis. Statistical analyses were done in R (version 3.2.0) as paired difference tests using Wilcoxon signed rank test. P-values <0.05 were considered significant.

Results: As of March 1st 2017, 61 patients have been included. In 33 cases, the time of intended follow-up has been reached. Four patients died prior to follow-up, including one patient who died before the acute imaging procedures. Four patients were excluded due to compliance problems. One patient was excluded due to disease downstaging resulting in omission of doxorubicin from the treatment plan. Of the 24 patients with complete data from both the baseline and late MR scans, 16 had lower LVEF values at follow-up: 0-5% (n=3), 6-10% (n=8), 10-15% (n=4) and >20% (n=1). Mean LVEF at follow-up was significantly lower (57.1%) compared to baseline LVEF (62.0%; p=0.01) and acute LVEF (64.3%; p=0.002). The LVEF decline from baseline to follow-up was paralleled by an increase in mean left ventricular end diastolic volume (LVEDV) of 10.0ml (p=0.03). Interestingly, an increase in LVEDV was already registered at the acute MR scan (7.3ml; p=0.03). The increase in LVEDV from the acute MR to follow-up was not significant. We also registered an acute increase of 7.4mL in mean stroke volume (p=0.02). However, from the acute MR to follow-up MR we found a significant decline in SV (p=0.02). There was no difference in SV from baseline to follow-up (p=0.7). The acute changes in LVEDV did not predict LVEF declines from baseline to follow-up (Figure 1).

Figure 1.

Summary/Conclusions: Our preliminary show that cardiac MR can be used for detection of declining LV function 1 year after doxorubicin exposure. It appears that cardiac MR may also provide information on acute functional changes in LVEDV and SV. We hope that our 82 Rb PET and 123I MIBG data will provide additional early signs of doxorubicin cardiotoxicity that can be used to predict subsequent development of HF.
Methods: Patients who met the following inclusion criteria were selected: i) histologic diagnosis of aNHL (diffuse large B-cell lymphoma, Burkitt lymphoma, and B-cell lymphoblastic lymphoma, peripheral T-cell lymphoma, anaplastic large cell lymphoma, NK/T-cell lymphoma, and T-cell lymphoblastic lymphoma); ii) patients who achieved CR after frontline or salvage chemotherapy with curative intent; and iii) time from the date of diagnosis to the date of last follow-up longer than 12 months. All patients in CR after frontline therapy were followed-up with clinical visits (symptom assessment, physical examination, and blood tests) every 1 to 6 months. Surveillance CT covering the neck, chest, or abdomen were performed every 3 or 6 months or when clinically indicated thereafter. The decisions regarding the surveillance CT were at the discretion of the treating physicians.

Results: Relapse was detected in 171 patients, of whom 52 had undergone surveillance CT during follow-up. Of these 152 patients, asymptomatic relapse was detected in 67 (44%) by surveillance CT and symptomatic relapse outside the surveillance interval was detected in the other 85 (56%). Detection of asymptomatic relapse by surveillance CT did not improve the overall or post-relapse survival in the relapsed aNHL patients. In addition, the interval of surveillance CT (3 or 6 months) did not affect survival. No subgroups were identified that favored the use of surveillance CT to detect relapse. Additionally, we analyzed the impact of surveillance CT in patients with refractory or relapsed aNHL who achieved CR after salvage chemotherapy (CR2). Of 315 aNHL patients relapsed/refractory to frontline chemotherapy (144 refractory and 171 relapsed patients), 99 patients achieved CR after salvage chemotherapy (18 refractory and 81 relapsed patients) and these patients were followed with clinical visits, with or without surveillance CT. Relapse was detected in 42 patients (42.4%). A total of 27 (64.3%) and 15 patients (35.7%) were identified as relapsed by methods other than CT scan and using surveillance CT, respectively. There was no significant difference in the median PFS between the two groups (12.5 months, 95% CI: 2.8 to 22.1 months vs 10.7 months, 95% CI: 0 to 14.5 months; p=0.182).

Summary/Conclusions: In conclusion, this study suggests that routine surveillance CT in aNHL patients for the detection of asymptomatic relapse might have a limited role in improving survival. Therefore, surveillance CT to identify relapse would only be recommended when relapse is clinically suspected.
Bone marrow failure syndromes incl. PNH - Biology

P226

IDENTIFICATION OF A NOVEL GERMLINE MECOM / EV1 VARIANT THAT RUNS IN A PEDIGREE WITH RADIOLNAR SYNDROSTOSIS AND AMEGAKARYOCYTIC THROMBOCYTOPENIA AND PREDISPOSSES TO ADULT ONSET MYELOID MALIGANCY

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Background: Radioulunar synostosis and amegakaryocytic thrombocytopenia (RUSAT), one of the rare bone marrow failure syndromes, is caused by a point mutation in HOXA11. In three simplex patients, de novo missense variants in MECOM have recently been reported as an alternative cause in individuals with RUSAT. MECOM, identified as a common ecotropic viral integration site 1 (EV1) in murine myeloid leukemia, is known as a key transcriptional regulator in hematopoiesis and is frequently involved in sporadic myeloid leukemia.

Aims: To screen for the causative genetic alteration in a family with four affected individuals out of three generations with radioulunar synostosis, incompletely penetrant congenital thrombocytopenia, hearing impairment due to dysplastic middle ear bones, patellar hypoplasia, and hand and foot dysmorpism. Notably, two of four affected individuals in our family developed adult onset myeloid malignancies (i.e. myelodysplastic syndrome (MDS) with excess blasts and MDS/myeloproliferative neoplasm-unclassifiable). No HOXA11 mutation was identified in this family.

Methods: Whole exome sequencing was performed in three affected individuals using a Nextera Rapid Capture kit and a NextSeq 500 instrument (illumina, Munich, Germany). Identified sequence variants were filtered for those that are de novo and only those carried by individuals in three generations with radioulnar synostosis were validated by Sanger sequencing.

Results: Following this approach, a novel MECOM missense variant (i.e. Cys766Gly, UniProtKB Q03112-1) was identified. The missense mutation affects a heavily conserved cysteine residue in C2H2-zinc finger motif 9 in the C-terminal zinc finger domain of MECOM. This residue is crucial for the tetrahedral coordination of a zinc ion stabilizing the zinc finger conformation and thus, is essential for DNA binding of the C-terminal zinc finger domain.

Summary/Conclusions: Our findings confirm the causality of MECOM missense mutations targeting the C-terminal zinc finger domain in subjects with RUSAT and indicate that MECOM needs to be considered in RUSAT pedigrees with no HOXA11 mutation. We report here for the first time that MECOM germline mutations are associated with an increased risk for adult onset myeloid malignancies. This extends the RUSAT-associated phenotype and proposes that MECOM germline mutations can cause a genetic predisposition to adult onset myeloid malignancy.

[BD and DS contributed equally to this work].
generated by ENU mutagenesis were characterized in terms of their hematopoietic and non-hematopoietic phenotypes during embryonic development and adulthood.

Results: The rad51 mutant fish developed key features of FA, including hypocalcemic kidney marrow (equivalent to mammalian bone marrow), sensitivity to crosslinking agents and decreased size. Interestingly, although mutants can survive to adulthood, they develop exclusively as sterile males. We show that some of the hematological symptoms stem from both decreased proliferation and increased apoptosis of embryonic hematopoietic stem and progenitor cells. Co-mutation of p53 was able to rescue the embryonic and adult hematopoietic defects seen in the single mutants, but led to early tumor development in the adult double mutants. We further establish that prolonged inflammatory stress can exacerbate the hematological impairment, leading to an additional decrease in kidney marrow cell numbers linked to excess p53 expression (Figure 1).

Figure 1. Example image of a p53, rad51 double mutant fish with a tumor behind the eye (A). Histological analysis showed the tumour to be a malignant peripheral nerve sheath tumor (B). The scale bar is 500 and 100µm respectively.

Summary/Conclusions: We demonstrate that zebrafish lacking functional rad51 is a viable and develop symptoms resembling FA. These findings strengthen the assignment of RAD51 as a Fanconi gene and provide more evidence for the notion that aberrant p53 signaling during embryogenesis leads to the hematological defects seen during later stages of life in FA patients. Further research on this novel zebrafish FA model will lead to a deeper understanding of the molecular basis of bone marrow failure in FA and the cellular role of the RAD51 protein.

P228

A NOVEL TELOMERASE RNA COMPONENT VARIANT IN A FAMILY WITH MACROCYTOSIS AND MILD VARIABLE CYTOPENIAS


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Background: Telomerase RNA component (TERC), encoded by the TERC gene, is an essential component of telomerase, a polymerase that adds the telomeric repeat to the 3’ lagging strand of DNA during cell replication. TERC variants have been causally associated with several hematological disorders, including autosomal dominant dyskeratosis congenita (DKC), aplastic anemia, myelodysplastic syndrome and acute leukemia, sometimes accompanied by non-hematological phenotypes. Here we report a likely pathogenic TERC variant associated with a hematological phenotype that predominantly affects the red cell lineage.

Aims: To describe the genotypic and phenotypic relationship of a new TERC variant.

Methods: Genomic DNA samples were analysed for sequence variants using the Oxford Red Cell Panel, a panel of 33 genes previously associated with human red cell disorders. Sanger sequencing was used to confirm the TERC variant. Telomere lengths were performed at the Laboratory for Molecular Haematology (LMH), Rayne Institute, Kings College Hospital.

Results: The index case AM (I.1) was a female who presented at age 56 with fatigue, and was noted to have a longstanding non progressive mild macrocytic anemia with very minimal thrombocytopenia. Further investigations (Table 1) revealed normal reticulocyte count, LDH, haematins, thyroid function, liver and renal function. Bone marrow aspirate demonstrated abnormal erythropoiesis with nucleo-cytoplasmic asynchrony, nuclear atypia, ragged cytoplasm, basophilic stippling and bi-nucleate forms. Granulopoiesis and megalakaryopoiesis were normal. The two daughters of I.1 also had abnormal blood counts and her paternal grandfather died of “pernicious anaemia”. None of the family have somatic features associated with DKC. The elder daughter (age 30) TW (II.1), had isolated lifelong macrocytosis and previous mild neutropenia (Table 1). The younger daughter (age 27) BM (II.2) had macrocytic anemia, thrombocytopenia (Table 1) and a recent pregnancy complicated by worsening thrombocytopenia, pre-eclampsia, placental dysfunction, liver dysfunction and foetal loss. Following delivery her liver function slowly returned to normal and a fibroscan was within normal limits. All three pedigrees with macrocytosis had a Chr3:16948268 (GRCh37) single nucleotide variant corresponding to a n.181A>C substitution in TERC (relative to transcript ENST0000002385.1), within the pseudoknot domain. Residue n.181 is highly conserved across mammalian species. This variant is absent from the gnomAD database of more than 230,000 TERC alleles, and the HGMD databases. The variant is within a TERC region in which previously reported variants have been associated with haematological phenotypes. In order to determine the pathogenicity of this variant, telomere lengths were assessed and found to be short in both Case I.1 and II.2 (Table 1). There were no other likely pathogenic variants in the Oxford Red Cell Panel genes. Together, these observations suggest that the n.181A>C substitution is causally associated with the macrocytosis phenotype.

Table 1.

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<tr>
<th>Parameter (normal range)</th>
<th>Haemoglobin (T20)</th>
<th>Mean cell haemoglobin (T20)</th>
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<tr>
<td>I.1 (index cases)</td>
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Summary/Conclusions: This report demonstrates a likely causal association between a newly identified TERC variant, short telomere length and a relatively mild hematological phenotype that is largely restricted to red cells. This emphasises the phenotypic heterogeneity associated with TERC variants, justifies the rationale of screening multiple genes simultaneously and suggests that TERC variant could potentially underlie a broader range of unexplained heritable blood cell abnormalities.

P229

GENERATION OF X-LINKED DYSKERATOSIS CONGENITA-LIKE HUMAN HEMATOPOIETIC STEM CELLS

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Background: X-linked Dyskeratosis congenita (X-DC) is an inherited syndrome caused by mutations in the DKC1 gene that encodes for the dyskerin nuclear protein. These mutations reduce the telomerase activity leading to premature telomere length attrition. Several organs can be affected in these patients, although the bone marrow failure (BMF) is the main cause of death in X-DC patients (more than 70% of cases). So far, the only curative treatment for BMF in DC patients is hematopoietic stem cell (HSC) transplantation. However, risks derived from conditioning regimes and the difficulties to find a compatible donor suggest that gene therapy may constitute a promising alternative in treating DC patients.
Aims: Because of the difficulties associated to the use of primary HSCs from DC patients for experimental studies, this study was focused on the generation of X-DC-like human HSCs by means of the down-regulated expression of dyserin in cord blood HSCs using different anti-DKC1 short hairpin RNAs (shRNA).

Methods: CD34+ cells were obtained by immunomagnetic purification from healthy human umbilical cord blood samples. These cells were then pre-stimulated with CD54 (ICAM-1) for two cycles of transduction with lentiviral vectors carrying both an anti-DKC1 shRNA and the puromycin-resistance gene. Transduced samples were then selected for 2 days with puromycin, and cultured in vitro or transplanted into immunodeficient NSG mice to evaluate the effects of shRNAs.

Results: Based on the inhibition of DKC1 gene expression, 3 shRNAs were selected among 7 designed shRNAs. Indeed, HSCs showed an inhibited telomerase activity, as well as a reduced clonogenic and hematopoietic reconstitution potential in NSG mice. Additionally, an increase in DNA damage and senescence was observed in DKC1-interfered CD34+ cells.

Summary/Conclusions: In vitro and in vivo data obtained from DKC1-interfered cells suggests that these cells can be used as a model of X-DC-HSCs. The generation of X-DC-like HSCs will facilitate the understanding of the molecular basis of the HSC defects characteristic of X-DC and contribute to the development of new experimental therapies for the treatment of the BMF of X-DC patients.

P230

STUDY OF EXTRACELLULAR VESICLES ROLES IN THE PATHOPHYSIOLOGY OF THROMBOSIS IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS DURING ECUILIZUMAB TREATMENT: A PILOT PROSPECTIVE LONGITUDINAL CLINICAL STUDY

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a disease characterized by complement-mediated hemolysis (Brodsky et al. Blood, 2007; Kelly et al. Br J Haematol, 2004; Hugel et al. Blood, 1999). Eculizumab, a human anti-C5 monoclonal antibody, is used in the treatment of PNH. Eculizumab has an impact on the amount and the pro-coagulant activity in PFP by STA®-Procoag-PPL assay and by thrombin cleavage of fibrinogen (PFP). We used mixed-effects linear regression (R 3.1.2 with nlme package) with six PNH patients treated with eculizumab. The study was led according to the declaration of Helsinki and approved by the local Ethic Committee. Informed consent was obtained for each patient. The aim was to measure, by flow cytometry and lymphocyte cytofluorometry (i.e. flow-FISH), the coagulation cascade (Owens et al. 2014). Inclusion criteria and reason for screening was either the clinical suspicion of the treating physician for a telomere maintenance disorder and/or the recommendations of the German Society of Hematology and Oncology (DGHO) published via Onkopedia. TL analysis of peripheral blood granulocytes and lymphocytes was carried out using combined fluorescence in situ hybridization and flow cytomtery (flow-FISH). Mutations in genes suspected to cause telomeropathies (i.e. TERT, TERC, DKC1, NOP10, NHP2, USB1, CTC1, RTFL1, TIN2, TCAB1) were analyzed by NGS using customized primer panels and amplicon-based sequencing on a Miseq sequencer (Illumina) in all patients with TL in lymphocytes below the 1% percentile of healthy controls.

Methods: 184 patients from 52 participating centers (80% academic centers) within Germany, Austria and Switzerland were screened for premature telomere shortening and included with informed consent into the ATR since November 2014. Inclusion criteria and reason for screening was either the clinical suspicion of the treating physician for a telomere maintenance disorder and/or the recommendations of the German Society of Hematology and Oncology (DGHO) published via Onkopedia. TL analysis of peripheral blood granulocytes and lymphocytes was carried out using combined fluorescence in situ hybridization and flow cytometry (flow-FISH). Mutations in genes suspected to cause telomeropathies (i.e. TERT, TERC, DKC1, NOP10, NHP2, USB1, CTC1, RTFL1, TIN2, TCAB1) were analyzed by NGS using customized primer panels and amplicon-based sequencing on a Miseq sequencer (Illumina) in all patients with TL in lymphocytes below the 1% percentile of healthy controls.

Results: Underlying initial diagnosis by the treating physician for PNH screening was aplastic anemia (AA, n=72, 39% of cases), unexplained cytopenia (UC, n=22, 11%); myelodysplastic syndrome (MDS, n=1, 0%), acute myelogenous leukaemia (AML, n=5, 3%) as well as other disorders (e.g. lung fibrosis, Diamond-Blackfan-Anemia, eto-dependent anemia, etc.). Median age of patients was 40.8 y (range 15 to 88 y). TL screening revealed 20% (38/184) patients with lymphocyte TL and 16% (30/184) of patients with granulocyte TL below the 1% percentile. NGS screening identified typical mutations associated with altered telomere maintenance in 15 out of 38 patients (40%) representing 8.2% of the total patient population. Median age of patients with mutations was 45.5 y (range 15 to 68 y). These mutations were detected in 7 out of 12 patients (6 of 7 patients (6/7) were treated with eculizumab and DKC1 (n=3). Mutations were observed in 5% of all AA, 12% of all UC, 50% of all FM-DKC, 13% of all SCCHN, 20% of all screened AML patients.

Summary/Conclusions: We provide the first analysis of a routine TL screening in PNH screening which revealed a modest number of patients with a decreased TL, and a heterogeneity in the initial diagnosis of PNH screening. A comprehensive analysis of the telomere screening for PNH screening however is of utmost importance given its significant clinical implications towards prognosis, treatment and family counseling.
Background: Mortality following HSCT in SAA pts over the age of 40 is reported to be in the order of 50%, without taking into account long term sequelae such as chronic GvHD, known to be more frequent in older patients. This has prompted international guidelines to recommend first line immunosuppressive therapy above 40 years of age. The question is whether this is still true in 2017.

Aims: Assess whether TRM in SAA patients grafted 2010-2015 is reduced, as compared to the era 2001-2009.

Methods: We used the WPSSA-EUBMT registry, and identified 748 pts aged 40 years or more, with acquired SAA, grafted between 2001 and 2015. We divided pts in 2 transplant eras:2001-2009 (n=327) and 2010-2015 (n=407). In the more recent period (2010-2015) pts were older (53 vs 49 year, p<0.01), were more often grafted from alternate donors (ALT) (64% vs 43%, p<0.01), with a greater use of BM (54% vs 41%, p<0.01), and with a longer interval dx-tx (317 vs 258 days , p<0.01), and more often received a fludarabine containing regimen (55% vs 42%, p<0.01).

Results: The overall survival 5 year survival of pts grafted in 2001-2009 was 57% , compared with 55% for pts grafted 2010-2015 (p=0.7). In multivariate analysis, including the interval diagnosis transplant, patient’s age, donor type, stem cell source and conditioning regimen, the lack of improved survival in 2010-2015 was confirmed (p=0.3). A very strong age effect was shown both in univariate and multivariate analysis: survival of pts aged 40-50 years, 51-60 years and >61 years , was respectively 64%, 54%, 41% (p<0.0001) and this was confirmed in multivariate analysis. The conditioning regimen , also proved to be a significant predictor, with improved survival for ALT transplants receiving FLU containing regimens (56% vs 46%, p<0.001). In general pts receiving either CY200 or a FLU containing regimen, did significantly better than pts receiving other preparative regimens (58% vs 50%, p=0.02). The use of a sibling donor (SIB) did not prove to predict survival in multivariate analysis. Pts receiving Campath in the conditioning , did significantly better than pts not receiving Campath (65% vs 54% p<0.01; similarly survival of patients with ATG was superior 59% vs 41% compared to patients not receiving ATG (p=0.01). When pts receiving either Campath or ATG (n=564) were compared to patients not receiving either (n=161), the difference in survival was 61% vs 41% (p<0.0001), and this was significant also in multivariate analysis. Combined primary and secondary graft failure was reduced from 16% to 12% in the two time periods (p=0.02), acute GvHD grade II-IV was reduced from 15% to 11% (p=0.01) and chronic GvHD was also reduced from 32% to 26% (p=0.01). Infections remain the leading cause of death in both transplant eras (18% and 22% respectively), followed by GvHD (5% and 4%) and graft failure (5% and 2%), whereas PTLD have been reduced from 3% to 0.5% (Figure 1).

Summary/Conclusions: HSCT in pts with acquired SAA aged 40 and over, continues to carry a significant risk of TRM also in 2010-2015, ranging from 36% in younger pts (40-50) to 59% in older pts (>60 years). Survival is predicted in multivariate analysis, by two crucial predictors: patients age and the use of either Campath or ATG, the latter giving a 20% survival advantage over no Campath/ATG. ALT and SIB donors produce similar survival. This study gives further support to current guidelines, suggesting first line therapy with ATG+CsA, in pts over the age of 40.

P233
CLINICAL AND GENETIC DIVERSITY IN DIAMOND-BLACKFAN ANAEMIA: AN UPDATE FROM THE UNITED KINGDOM
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Background: Diamond-Blackfan anaemia (DBA) is an inherited bone marrow failure syndrome (BMFS) caused by mono-allelic, loss-of-function mutations in ribosomal protein (RP) genes. DBA is rare and has a wide spectrum of clinical manifestations, hence the utility of patient registries.

Aims: We evaluated the clinical and genetic spectrum of DBA in a large cohort of patients in the UK, aiming to identify novel features of the disease.

Methods: We performed a retrospective analysis of data from 103 confirmed cases of DBA, including 4 multiplex families. All living patients had undergone a bone assessment at our specialized centre in the last 5 years. Data were collected from family interviews, patient records and referring clinicians.

Results: The 103 patients with DBA were born in a 48-year period (1967-2015), i.e., an incidence of 3 per million live births. Demographic and clinical characteristics are shown in Table 1. NGS analysis of 80 RP genes plus GATA-1 identified pathogenic mutations in 71% of cases and 7 putative novel mutations, currently undergoing validation. To date, mutation screening of both parents has been performed in 32 families with DBA. Twenty-five mutations are sporadic while 7 are autosomal dominant; in 3 of the latter, the parent is a silent ‘carrier’ without anaemia. In one case of an affected child, the causative mutation was detected in the peripheral blood of both parents but was present in 7/22 embryos generated for in vitro fertilisation, suggesting germline mosaicism. 80.5% of cases in our cohort presented within the first year of life. For the first time we report a high rate of perinatal problems in DBA. Prematurity +/- intrauterine growth restriction (IUGR) occurred in 31/87 (35.6%) of evaluable patients. Specific abnormalities included: hydrops fetalis (3/87), prematurity (22/87) and IUGR (16/87). In addition to congenital anomalies classically associated with DBA, we identified abnormalities of the spine and axial skeleton in 9.2% of patients. These did not correlate with a particular genotype. Our cohort exhibited multiple comorbidities, including some not previously reported to be associated with DBA: hematosis (10.7%), neuropsychiatric (17.4%) and gastrointestinal (GI) disorders (25.7%). These complications were not associated with particular treatment regimens. In terms of the natural history of DBA, a lower proportion of our patients (22%) than previously reported in the literature (40%) were able to maintain a normal Hb on long-term steroids. Three patients failed therapy and were treated with busulfan. In addition to congenital anomalies (MDS, B- ALL, BCC and cervical intraepithelial neoplasia) in 4 different patients. The lower incidence in our cohort compared with that reported by the North American DBA registry may be explained by differences in the median ages of the 2 cohorts (12y versus 18y, respectively) and the shorter follow-up of our patients.

Table 1.

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Summary/Conclusions: This retrospective analysis of the UK’s DBA cohort confirmed several findings from other registries but also revealed novel features, including a high prevalence of i) premature birth and neonatal complications ii) abnormalities of the axial skeleton and iii) neuropsychiatric disorders. Prospective longitudinal studies are warranted to better characterise these co-morbidities.

Materials and Methods: We followed the guidelines for reporting clinical features in DBA, a large retrospective analysis of 103 confirmed cases, including a metoclopramide trial. In total there were 4 incidents of malignancy (MDS, B-ALL, BCC and cervical intraepithelial neoplasia) in 4 different patients. The lower incidence in our cohort compared with that reported by the North American DBA registry may be explained by differences in the median ages of the 2 cohorts (12y versus 18y, respectively) and the shorter follow-up of our patients.

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P234

BONE MARROW FAILURE SECONDARY TO NOVEL/KNOWN PRIMARY IMMUNODEFICIENCY-RELATED MUTATIONS. A SINGLE CENTER ANALYSIS

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Background: Differential diagnosis between acquired and congenital forms of Marrow Failure (MF) has always represented a crucial point in the diagnostic work-up, since genetic forms do require a different therapeutic approach. It is also known that patients with congenital MF may also show immunodeficiency that, in some cases, can represent the first/ or revalent sign of the disease and therefore can be misinterpreted as a Primary Immunodeficiency (PID). On the other hand, patients with PIDs may also show MF as a result of an immune-mediated attack of marrow precursors thus generating a phenotypic overlap that can impair the correct diagnosis.

Aim: In this report we analyzed all patients with MF evaluated in our Unit with the aim to identify the type and incidence of underlying molecular defects, in particular those related to PIDs.

Methods: We retrospectively evaluated all diagnosis performed in patients with single/multi-lineage MF followed in our Unit. DEB test was used to screen Fanconi Anemia (FA). Other congenital MFs have been searched by Sanger and/or NGS molecular analysis depending on the available tools over the years.

Results: Between 2009-2016, 88 patients have been studied for single-lineage (25) or multilineage (63) MF. 48 (64%) were classified as having an underlying PID.

Table 1 shows clinical characteristics and mutations of patients with PIDs.

Table 1.

Summary/Conclusions: This report shows that patients presenting with single/multi-lineage MF may have an underlying PID in a considerable number of cases. We also show that MF represented the most relevant clinical sign in patients with PI3KCD, TACI, or CD40L mutations, thus widening their clinical phenotype. We conclude that an accurate immunological work-up should be performed in all patients with MF and that PIDs-related genes should be included in the molecular screening of MF in order to identify specific disorders that may potentially receive targeted treatment and/or the appropriate conditioning regimen for SCT.

P235

COVERSIN, A NOVEL C5 COMPLEMENT INHIBITOR, FOR THE TREATMENT OF PNH: RESULTS OF A PHASE 2 CLINICAL TRIAL


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Background: Paroxysmal nocturnal haemoglobinuria (PNH) leads to episodic haemolysis secondary to an acquired deficiency of PIGA anchor molecules on the surface of erythrocytes which play a critical role in protecting the cells from complement mediated lysis. Until the advent of eculizumab, a monoclonal antibody which prevents the cleavage of C5 to C5a and C5b, PNH was associated with considerable morbidity and a poor long-term prognosis. However, eculizumab needs to be administered by health care professionals by intravenous infusion which may interfere with the life-styles, occupations and personal privacy of patients and the interval dosing has led to concerning breakthrough haemolysis. Coversin is a protein suitable for small-volume subcutaneous (SC) injection which can be self-administered by patients.

Aim: The aim of this study is to investigate the safety and efficacy of the complement C5 inhibitor Coversin in the treatment of PNH.

Methods: A Phase 2 single arm open label trial of Coversin is currently ongoing under which patients, either newly diagnosed with PNH or who have not previously had access to complement inhibitors, are treated for 90 days. Coversin is supplied as a lyophilised powder, reconstituted with water for injection to give a buffered aqueous solution of Coversin 30mg/mL. The trial population consists of up to 10 adult patients with a diagnosis of PNH confirmed by flow cytometry. Treatment commences with an ablating regime (AR) consisting of a fixed dose of 60mg followed by 3 doses of 30mg q12 hours delivered by SC injection. Following the AR, a dose of 15mg q12 hours is given for a further 26 days when, if the patient’s disease is well controlled, they switch to 30mg q24 hours for the remainder of the trial. The dose can be increased by two incremental steps according to a pre-specified algorithm for patients not satisfactorily controlled on the basis of serum lactate dehydrogenase (LDH) or clinical grounds at any time during the 90-day period. The primary endpoints are safety and reduction of serum LDH to ≤1.8 X the upper limit of normal (ULN) for the local laboratory. Secondary endpoints include LDH at 28, 60 and 90 days, terminal complement activity assessed by CH50 ELISA (Quidel®), sheep erythrocyte haemolysis assay. PK (free and bound Coversin levels), anti-drug antibodies (ADA) and quality of life.

Results: The trial is still ongoing and has currently enrolled 5 patients, four of whom remain on Coversin. Three patients have required single dose increases during the initial 28-day period, one of whom was later withdrawn when a morbidity was suspected. Two patients have moved to a single daily dose. Updated results of these and any patients enrolled subsequently will be presented. To date 2 patients have achieved the primary efficacy endpoint, two have not yet reached the 28-day point. There have been no serious or significant adverse events and the drug has been well-tolerated. A few mild injection site reactions have been recorded but these appear to diminish with time. There has been no evidence of the formation of neutralising antibodies.

Summary/Conclusions: It currently appears that treatment with Coversin is safe and effective in controlling hemolysis in PNH and that patients are capable of self-administering the drug. Coversin may be an effective alternative for patients with PNH who prefer the independence of self-administration. The relatively short dose interval may also help to reduce breakthrough events due to trough levels of drugs administered at two weekly intervals or longer.
**Background:** CLL is a highly heritable cancer. Although GWAS have identified ~30 independent SNPs associated with CLL, these are estimated to account for only ~19% of the inherited component of CLL.

**Aims:** We hypothesized that this missing heritability might arise from rare coding variants (MAF <0.01), and sought to identify these through an exome-wide association study comparing rare germline variants between CLL patients and controls.

**Methods:** We investigated 516 CLL patients of European descent who were compared to 8,920 ethnically matched, non-cancer population controls. CLL cohorts included 235 CLL patients from DFCI (128 previously reported, 107 unpublished exomes), and 281 CLL patients enrolled on the CLL8 trial of the German CLL Study Group (WES data reported previously). An additional 130 CLL patients in an extension cohort included 24 from our published whole-genome sequencing study and 106 from an early publication of the IGCG. Non-cancer controls came from 3 sources: 2,520 from the 1000 Genomes Project; 6,852 from the Exome Sequencing Project; and 7,611 from a collaborative study of genetic cohorts: 235 CLL patients from DFCI (128 previously reported, 107 unpublished exomes), and 281 CLL patients enrolled on the CLL8 trial of the German CLL Study Group (WES data reported previously).

**Results:** Using an unbiased, gene-based rare variant association analysis comparing cases to controls, we identified two genes significantly enriched for rare coding variants in CLL cases: CDK1 and ATM (OR 5.8, 95% CI 3.5-9.8; 95% CI 2.9-13.1). One recurrent missense variant, CDK1 p.R59C, observed in 5 cases and 0.3% of controls, was predicted to be damaging by PolyPhen2 and deleterious by the SIFT tool, and is driving the association. The second significant gene was ATM, in which we found a total of 112 cases carrying 52 distinct rare germline variants and 111 recurrent rare variants in ATM (15.7% vs. 6.5%, OR = 2.5; 95% CI = 1.9-3.2). The majority of recurrent rare variants in ATM were non-synonymous missense variants, with L230T being the most enriched (2.3% cases, OR=1.0, 4.9-20.7). Subsequent validation in 149 independent CLL cases revealed a similar frequency of 2.0% (3 out of 149) of the L230T variant. We then added 130 CLL cases and performed an expanded joint analysis, which has been shown to improve the statistical power of detecting genetic associations compared to a matched, unrelated samples over DNA sites with sequencing coverage sufficient for only 19% of the inherited component of CLL.

**Summary/Conclusions:** We conclude that PRO-seq and dREG analysis identifies evidence of active differential transcription based on genotype in the region of 5 out of 6 GWAS-identified SNPs that we have investigated so far.

**Background:** Genome-wide association studies (GWAS) have identified multiple loci that are statistically associated with CLL susceptibility. These single nucleotide polymorphisms (SNPs) are primarily located in non-protein coding genomic regions. Data suggest that these variants are enriched in regulatory elements.

**Aims:** We tested the hypothesis that CLL risk variants are in or near regulatory elements that influence nearby target genes.

**Methods:** To investigate SNP allele-specific impacts on gene expression, we selected 15 SNPs from 13 loci that achieved genome wide significance in initial CLL GWAS studies. We investigated whether the published GWAS SNP (if present on the Affymetrix 6.0 SNP array) or proxy SNP(s) chosen using the SNP Annotation and Proxy Search (SNAP) software, based on their high linkage disequilibrium (LD) (r²>0.68) with the selected GWAS SNP. Genotype elements were determined in tumor (n=143) and saliva (n=79) DNA from CLL patients (who had provided written informed consent) and tumor and saliva DNAs were concordant in at least 96% of cases (except rs477184 at 92%). Given the high concordance with saliva, which is likely related to the stable genome of CLL, SNP genotypes from tumor samples were used for the analysis in order to significantly increase our sample size. Allele-specific gene expression was then evaluated for these samples by using Affymetrix Illumina U133 Plus 2.0 array gene expression data, focusing on genes within 1 Mb in either direction from a given SNP. In order to elucidate whether these associations were due to functional effects on transcription, we used a novel assay called precision run-on sequencing (PRO-seq). PRO-seq efficiently maps active transcription regulatory elements and provides quantitative and directed maps of transcriptionally-engaged RNA polymerases. The algorithm, discriminatory regulatory-element detection from GRO/PRO-seq (dREG), is then used to predict the presence of TRES from raw PRO-seq data, allowing for identification of functional elements in the vicinity of SNPs and quantification of their allele-specific effects on enhancer activity and gene transcription.

**Results:** Our gene expression analysis demonstrated 6 significant SNP-gene associations: rs674313 (6p21.3) with HLA-DQA1 (p <0.0001), rs872071 (p=0.009), rs477184 (15q23; proxy for rs1765083) with TLE3 (p<0.009), rs783540 (15q25.2) with CPEB1 (p=0.01), rs305088 (16q24.1; proxy for rs305061) with CDK14/MAMDC8 (p=0.03) and rs4902322 (18q13.32; proxy for rs11083846) with FKRP (p<0.0001). Two associations were successfully validated in a completely independent gene expression replication analysis (n=54): rs674313 with HLA-DQA1 (p<0.0001) and rs477184 with TLE3 (p=0.0116). To annotate candidate regulatory elements, we evaluated transcription level at or near all six significant functional associations in the initial gene expression analysis in a cohort of 12 CLL samples. Transcription level at or near 3 SNPs (rs674313, rs477184, rs305088) correlated with genotype in a dose dependent manner. When we expanded the analysis to the entire region of LD around each SNP, we were able to demonstrate a dose-dependent effect in all SNPs in 6-7 out of 10 analyzed samples (rs477184 p=0.027).

**Summary/Conclusions:** We conclude that PRO-seq and dREG analysis identifies evidence of active differential transcription based on genotype in the region of 5 out of 6 GWAS-identified SNPs that we have investigated so far.
17p13 deletions were assessed by FISH (MetaSystems). More than a half of the cohort (57%) was sequenced using ultra-deep NGS for TP53 exons 2-11. Genome-wide analysis was performed on CytoScanHD arrays (Affymetrix) and correlated to conventional cytogenetics (CpG/IL-2 stimulation).

**Results:** Out of the cohort positive for TP53mut, 72/200 patients (36%) harbored single dominant TP53mut without del(17p). We selected 43 of these cases with variant allele frequencies (VAF) >10% for CytoScan analysis to explore the potential presence of 17p cn-LOH. In 42% (18/43) of the cases, cn-LOH in 17p locus was detected in a proportion of CLL clone correspondingly to the TP53 VAF (median TP53 VAF 59.4%, range 12.9–99.9%). In 3/43 cases, heterozygous deletion previously undetected by FISH was newly revealed. Thus, the truly monoallelic mutations were confirmed in 23/43 cases, where by no cytogenetic abnormality in 17p locus was observed (median TP53 VAF 43.5%, range 10.5–51.3%). Applying a VAF cut-off of 55% indicating fully expanded heterozygous mutation (taking into account the potential unequal representation of forward and reverse strands in NGS data), 7/29 (24%) cases below the cut-off still harbored 17p CNLOH. These results show that it is not possible to use an arbitrary VAF cut-off (>50%) to identify biallelic mutations due to cn-LOH. When we compared genomic complexity of leukemia clones with monoallelic vs biallelic TP53mut as determined by the CytoScan array, the latter group exhibited significantly more genomic abnormalities (p=0.0388) and also preference for different recurrent chromosomal abnormalities (p=0.0001; 17p locus excluded from this analysis). However, there was no significant difference in overall survival between the groups (p=0.5856).

**Summary/Conclusions:** cn-LOH in 17p locus is present in approximately half of the patients with single dominant TP53mut and results in biallelic TP53 gene inactivation despite the absence of del(17p); truly monoallelic TP53mut gene mutations with an intact second allele occur in CLL with comparable frequency. Although 17p cn-LOH is associated with increased genomic instability, it does not have worse impact on clinical outcome than truly monoallelic TP53mut.


**P239**

**INTERGRATED Oligo/SNP ARRAY- AND NEXT GENERATION SEQUENCING BASED ANALYSIS IS REQUIRED TO DETERMINE TP53/17P STATUS IN CLL PATIENTS**

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**Background:** 5–cell chronic lymphocytic leukemia (CLL) exhibits a highly heterogenous clinical course, with overall survival rates varying from several months to decades. Mutation status of the IGHV genes and specific genomic abnormalities, such as deletion of 11q22 on loss of the 13q14 region provide prognostic information. However, more importantly deletion of 17p and/or the presence of a TP53 mutation, which are both associated with a poor prognosis identify a CLL patients with the highest risk of progression. Recently clinical trials with tyrosine kinase inhibitors such as ibrutinib and idelalisib have demonstrated decreased progression with an intact second allele occur in CLL with comparable frequency.

**Aims:** To determine whether CCE occurs during ibrutinib therapy and at disease progression.

**Methods:** We analyzed 336 pts treated on investigational studies with ibrutinib or idelalisib plus rituximab for CLL. In pts who progressed, we analyzed FISH and next generation sequencing (NGS) results pre-treatment and at progression, to identify CCE. Additionally, we identified a sub-group of 97 relapsed/refractory pts who had serial FISH analysis performed in bone marrow ≥1 year apart, to determine whether there were significant changes in sub-clonal composition of CNAs detected by FISH during treatment in the absence of disease progression.

**Results:** In total, 37 of 336 pts (11%) progressed during ibrutinib-based therapy. Of these pts, 15 had FISH analysis both pre-treatment and at progression: pre-treatment, 10 had del(17p), 1 had del(11q) and 4 had isolated del(13q). All 4 of the pts with isolated del(13q) pre-treatment had new sub-clonal TP53 abnormalities detected at progression: two pts had del(17p), 1 had del(11q) and 1 pt developed two additional copies of 13q at progression. The pt with del(11q) pre-treatment who progressed developed Richter Transformation (RT) in the bone marrow at progression, without either del(11q) or del(17p) identified by FISH, suggesting that the RT arose from a common ancestral clone without del(11q) or del(17p) identified by FISH, suggesting that the RT arose from a common ancestral clone without del(11q) or del(17p) identified by FISH, suggesting that the RT arose from a common ancestral clone without del(11q) or del(17p) identified by FISH. Notably, in responding pts, there was no expansion of high-risk sub-clones with biallelic del(13q) in two patients who initially had monoallelic del(13q). In 4 pts, CCE was identified at progression, including 17 new abnormalities in one pt. All 4 pts had complex karyotype and del(17p) by FISH pre-treatment and 3 of 4 had evidence of multiple, related, complex sub-clones pre-treatment. Figure 1 shows inferred clonal evolution pattern for one pt.

**Figure 1.**
Summary/Conclusions: Emergence of high-risk clones containing del(17p) and or del(11q) may be seen at disease progression in ibrutinib-treated patients. Analogous to allelic expansion of TP53 mutations after chemotherapy, we hypothesize that small del(17p) or del(11q) subclones were present prior to therapy in these pts, below the sensitivity of existing FISH techniques and expanded under the selective pressure of ibrutinib treatment. Development of a more sensitive technique to identify small sub-clones with SFA del(17p) may therefore be important. Additionally, complex CCE occurred at progression in several cases, indicating genomic instability and potentially contributing to therapeutic failure.

Pairwise association showed statistically significant co-occurrence between tri(12) and mutations in KARS/BCOR (both <0.05), NOTCH1 mutation and ZMYM3 (p=0.01), SPEN (p=0.05) mutations, and TP53 mutation and del(17p) (q <0.01). Complex karyotypes (q <0.05). When correlating with clinical response to lenalidomide, worse overall response (OR) in the untreated group was associated with del(17p) (p=0.019) and KRAS mutation (p=0.05), whereas as mutation in SF3B1 (p=0.025), MGA (del(13q), DDXX (p=0.035), DDYX (p=0.001), complex karyotype (p=0.035) and del(17p) (p=0.031) were associated with worse OR in R/R group. In the untreated group, del(17p) and TP53 were associated with worse progression-free (PFS) (p=0.002 and 0.003, respectively).

R/R cohort, complex karyotype, del(17p) and mutations in SF3B1 and TP53 were associated in patients not responsive to lenalidomide. If TP53 was associated with worse PFS but not OS (refer to provided Figure 1). In one of the multivariate models, SF3B1 (P=0.005) mutation and having TP53 or del(17p) (P=0.02) were prognostic for survival in R/R cohort.

Summary/Conclusions: Tumor mutational heterogeneity in CLL is due to intrinsic tumor biology and selective drivers from previous treatments, which can then affect response and survival in lenalidomide-based therapies.

P241
LANDSCAPE OF SOMATIC MUTATIONS AND THEIR IMPACT ON RESPONSE AND OUTCOMES FROM LENALIDOMIDE-BASED THERAPIES IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: Lenalidomide, either as a single agent or in combination with anti-CD20 monoclonal antibody, is clinically active in CLL and offers durable response in some pts. Predictive and prognostic impact of somatic mutations are not well known in pts with CLL who have received lenalidomide-based therapies.

Aims: Investigate the overall landscape of CLL gene mutations in both previously untreated and relapsed/refractory (R/R) pts. Determine associations between CLL gene mutations and clinical characteristics. Establish predictive and prognostic impact of CLL mutations in the context of lenalidomide-based therapies.

Methods: In the 288 pts with CLL who were treated in one of the lenalidomide-based clinical trials at our institution, we performed targeted gene capture exome sequencing of 295 genes that have been recurrently mutated in hematologic malignancies on pre-treatment samples. This sequencing platform also included more than 1000 cyt SNP position that allowed copy number variation (CNV) estimation. We used Mutect and Pindel algorithms to call high-confidence somatic mutations and used in-house algorithm to detect copy number variations (CNVs) in CLL samples.

Figure 1.

Results: Among the 288 CLL pts treated with lenalidomide, 102 (35%) were previously untreated and 186 had R/R CLL. Ninety two patients (32%) received lenalidomide as a single agent and 196 patients (68%) received in combination with anti-CD20 monoclonal antibody, is clinically active in CLL and offers durable response in some pts. Predictive and prognostic impact of somatic mutations are not well known in pts with CLL who have received lenalidomide-based therapies.

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Results: Among the 288 CLL pts treated with lenalidomide, 102 (35%) were previously untreated and 186 had R/R CLL. Ninety two patients (32%) received lenalidomide as a single agent and 196 patients (68%) received in combination with rituximab or ofatumumab. In total, we detected 470 high-confidence somatic mutations in 61 genes in 281 pts (76%). In addition to TP53 and tri(19). The most frequently mutated gene was SF3B1 (15%), followed by NOTCH1 (14%) and TP53 (14%) with 13 gene mutations occurring ≥3%.

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FAILED HYDROXYMETHYLATION CONTRIBUTES TO A CHRONIC LYMPHOCYTIC LEUKEMIA SPECIFIC EPIGENOTYPE
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Background: During normal hematopoiesis, a coordinated epigenetic and transcriptional programming is necessary to achieve lineage development. B cell differentiation is predominantly related to loss of DNA methylation at the enhancers and promoters of B cell-specific genes; e.g. transcription factors (TFs). In chronic lymphocytic leukemia (CLL), failure of proper epigenetic programming contributes to deregulation of B cell transcriptional programs and results in CLL phenotypes with highly variable outcomes. The mechanisms leading to failed epigenetic programming and to establishment of a CLL epigenome are not well understood. Genomic sites of failed epigenetic programming coincide with binding sites of key B cell TFs. Active DNA demethylation through TET-dioxygenase mediated conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) and subsequent products is one of the mechanisms involved in physiological epigenetic B cell programming, and deregulation of this process could contribute to establishment of the CLL epigenome.

Aims: Here, we investigated the role of TET2-mediated DNA demethylation through differential 5hmC acquisition in healthy and in CLL B cells. We further studied mechanisms and TFs involved in regulation of 5hmC conversion during CLL pathology.

Methods: Clonal B cell specimens from 122 CLL patients were subjected to DNA methylation profiling using Illumina 450k arrays. 17 CCL and 4 healthy B cell samples (CD19+) were used for DNA methylation profiling using Illumina Epic arrays and for hydroxymethylated DNA immunoprecipitation (hMeDIP) using a monoclonal 5hmC mouse antibody and the NEBNext Ultra DNA Library Prep Kit for analysis on a Illumina HiSeq2000 sequencer. Global 5hmC levels were quantified by dot blots. TET2, and EBF1 mRNA and protein expression was evaluated by qPCR and Western Blot, respectively.

Results: By dot blot, we found decreased 5hmC levels in CLL as compared to CD19+ B lymphocytes. 5hmC was further reduced in IGHV unmutated compared to IGHV mutated CLL patients. To identify distinct regions with gain or loss of 5hmC, we performed genome-wide 5hmC profiling by hMeDIP. We confirmed a significantly lower number of hydroxymethylated peaks in CLL (137114) compared to HBC (249421) which remained stable when separating to good-prognostic (IGHV or ip-IGHV unmutated, del(11q), p<0.0161) patients (defined by the IGHV mutation status, Rai/Binet stages, CD38 positivity, del(11q) and del(17p)). Differential binding analysis (DBA) revealed 5988 significantly differentially hydroxymethylated reads between CLL and HBC samples (FDR<0.05). Pathway analysis showed that regions which lost hydroxymethylation in CLL were involved in B cell receptor (BCR), Class I PI3K, CXCR-4, c-Mec and IL3 signaling. To further identify mechanisms that are involved in failed hypomethylation and 5hmC loss in CLL, we aimed at profiling these subsets match to the recently proposed epigenetic classification of CLL, broadly divided into i) naïve like CLL (n-CLL), ii) good-prognostic, memory like CLL (m-CLL), iii) a third intermediate CLL subgroup (i-CLL), which have borderline mutated IGHV genes and an intermediate outcome. For this purpose, we utilized the same GoGens database to classify CLL patients as either i-poor-prognostic, naive like CLL (n-CLL), ii) good-prognostic, memory like CLL (m-CLL), broadly corresponding to IGHV unmethylated and mutated CLL, respectively; and iii) a third intermediate CLL subgroup (i-CLL), which have borderline mutated IGHV genes and an intermediate outcome. Of these, 82K, p=not significant). This implies that subsets #1 and #2 have a higher epigenetic burden than n-CLL, which is in line with the more aggressive disease seen in these two subsets compared to the broader category of n-CLL patients. Focusing on subset #2, we observed that almost all cases clustered separately from i-CLL in supervised clustering analysis, providing further support that subset #2 forms a distinct subgroup of i-CLL. Subset #2 cases frequently carry del(11q) and harbor SF3B1 mutations, however, neither the IGHV mutation status nor the presence of del(11q) or SF3B1 mutations had any impact on the epigenetic burden within subset #2.

Summary/Conclusions: Stereotyped CLL subsets differed significantly in their methylation profiles. That said, subset #1 and #4 clustered at large with n-CLL and m-CLL categories, respectively, implying common cellular origin. In contrast, subset #2 emerged as the first defined member of the i-CLL group, which in turn alludes to a distinct cellular origin for subset #2 and i-CLL patients. Both subsets #1 and #2 displayed a higher epigenetic burden compared to n-CLL and i-CLL, respectively, which is likely reflected in the very poor outcome associated with these two subsets.
Chronic lymphocytic leukemia and related disorders - Clinical

**P245**

**ADDING OBLINUTUZUMAB TO IBRUTINIB ENHANCES DEPLETION OF CLL CELLS IN PERIPHERAL BLOOD AND BONE MARROW AFTER 1 & 6 MONTHS COMBINED THERAPY INITIAL RESULTS FROM THE BLOODWISE TAP ICICLLe EXTENSION STUDY**

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**Background:** A major aim of CLL treatment is to eradicate detectable minimal residual disease (MRD). Ibrutinib is an effective treatment for CLL that results in immediate lymphocytosis persisting in most patients for several months. Obinutuzumab is a second-generation anti-CD20 monoclonal antibody which can effect rapid resolution of lymphocytosis and eradication of MRD in some CLL patients. The ICCLIle Extension Study expands on the ICCLIle trial (ISRCTN12695354) to examine the efficacy and safety of the combination treatment of obinutuzumab and ibrutinib.

**Aims:** The ICCLIle trial was a single-arm, multicentre feasibility study that recruited 40 patients with CLL requiring treatment, 20 treatment-naïve (TN) and 20 relapsed/refractory (RR), to receive continuous ibrutinib therapy until confirmed MRD negative remission (<0.01% residual disease) or disease progression. The ICCLIle Extension Study is the proportion of patients achieving MRD-negative remission by IWCLL criteria (depletion of CLL below 0.01% in the peripheral blood and bone marrow) at or before 9 month assessment.

**Methods:** The Events are collected from registration until 30 days after end of treatment and reported using the Common Terminology Criteria for Adverse Events v4.0. MRD was assessed by multiparameter flow cytometry according to ERIC 2016 guidelines with a detection limit ≤0.004%.

**Results:** 31 patients (22 ibrutinib-naive and 9 pre-treated) are evaluable for response assessment after 1 month of combination treatment. There have been no reports of tumour lysis syndrome within the first month of combination treatment. There were 2 separate reports of grade 2 infusion related reactions, both on day 1 of obinutuzumab. In the 22 ibrutinib-naive cases peripheral blood (PB) CLL counts remained at or below baseline levels in 17/22 cases from week 1 onwards. After 1 month of combination therapy the PB CLL count was a median 31% of baseline levels (range <1%-174%) compared to median 215% (range 29%-3570%) for RR patients on ibrutinib monotherapy. Percentage CLL cells in the bone marrow (BM) aspirate after 1 month of combination therapy reduced from a median 83% (range 23-94%) to a median 47% (range 5-85%, P=0.003, Wilcoxon matched-pairs signed ranks). For RR patients on ibrutinib monotherapy there was no change in BM at 1 month; baseline median 85% (range 11-96%) compared to median 86% (range 50-98%, P=0.96). Changes in BM aspirate CLL percentage were confirmed by morphological assessment of a trephine biopsy with all evaluable patients receiving obinutuzumab showing improvements in the cellularity and/or extent of infiltration. BM assessment at 1 month was not mandated for the 9 pre-treated patients but all showed decreased PB CLL counts with 4/9 achieving <0.01% residual disease within 3 months of starting obinutuzumab. 13 patients have completed 6 months of obinutuzumab treatment with marrow assessment at 9 months showing a further 21 log depletion in CLL percentage in 9/13 patients with 4/6 pre-treated patients achieving <0.01% residual disease.

**Summary/Conclusions:** The data indicate that for RR patients, the addition of obinutuzumab to ibrutinib results in a substantial improvement over ibrutinib monotherapy in the depletion of CLL cells from peripheral blood and bone marrow after 1 month of combination therapy, and continued improvement after 6 months combination therapy, with MRD-negative BM responses for patients who have had >1yr prior ibrutinib monotherapy. Residual disease levels in the BM after the 6 months of combination treatment will be available for 25 participants by June-2017.

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**P246**

**CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS EXPRESSING THE LIGHT CHAIN IGLV3-21 OR THE IGHV MUTATIONAL STATUS**

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**Background:** The immunoglobulin heavy-chain gene (IGHV) mutational status is currently considered the gold standard of prognostic in Chronic Lymphocytic Leukemia (CLL): unmutated (UM) immunoglobulin heavy chain region (IGHV) is associated with poor prognosis while patients with mutated IGHV (M) have more indolent disease. An exception are patients with IGLV3-21/IgL3-21 who have poor prognosis irrespectively of the IGHV mutational status. Interestingly, IGLV3-21 is co-expressed with IGLV3-21 in the majority of cases.

**Aims:** Here we aimed to study the impact of the light chain IGLV3-21 on CLL prognosis. This light chain has never been characterized independently of the heavy chain IGHV3-21.

**Methods:** On 405 CLL patients from 3 independent cohorts (A. an initial cohort of 32 patients with aggressive CLL, and 2 cohorts of CLL patients where samples were obtained at diagnosis (B: n=270 and C: n=103), we analyzed the impact of the presence of IGLV3-21 on treatment-free (TFS) and overall (OS) survival. IGLV3-21 positivity was determined by real-time PCR and confirmed by Sanger sequencing.

**Results:** Among the 32 patients with aggressive CLL, we found that 9 (28%) patients who had an IGLV3-21 rearrangement, but only 1 patient carried the heavy chain IGHV3-21: IGLV3-21 patients had a median TFS of 17 months compared to 44 months in patients with another light chain (P=0.0270). Similarly, IGLV3-21 patients had a shorter median OS (88 months vs >192 months, P=0.0287). We verified these results in 2 independent cohort obtained at diagnosis. In cohort B (n=270), 30 (11%) expressed an IGLV3-21 light chain and 10 (4%) an IGHV3-21 (of which 8/10 also carried the light chain IGLV3-21 rearrangement). Patients with IGLV3-21 had a median TFS/OS of 29/183 months compared to patients without IGLV3-21 who had a median TFS/OS of 88/292 months (P=0.0003,P=0.0142). In cohort C (n=103), 9 (9%) expressed an IGLV3-21 light chain but only 1 (1%) had a heavy chain IGHV3-21. In this cohort, IGLV3-21 patients had a median TFS of 21 months not statistically different from UM M patients (28 months) while IGHV3-21 M patients had a median TFS of 93 months (P<0.0001). We then pooled the 3 populations (n=405) in order to increase the under-represented subgroups and analyzed the association of the IGLV3-21 with the IGHV mutational status: patients with either IGLV3-21 or IGLV3-21 (with a M or UM IGHV) displayed a prognosis similar to UM patients: median TFS was 129, 48, 36, 24, 23 months for M, IGLV3-21/M (P=0.0005), UM (P<0.0001), IGLV3-21/UM (P<0.0001) and IGHV3-21 (P<0.0001) patients, respectively (Figure 1A). Similar results were observed for OS with a median OS of 292, 88, 174, 90 and 183 months M, IGLV3-21/M (P<0.0001), UM (P<0.0001), IGLV3-21/UM (P<0.0001) and IGHV3-21 (P<0.0001) patients, respectively (Figure 1B). If all IGLV3-21 (n=48) were considered independently of their heavy chain, IGLV3-21 median TFS (24 months) was similar to UM patients (36 months, P=0.5824) and statistically different from M patients (129 months – P<0.0001, Figure 1C). Similar results were observed for OS (Figure 1D).

**Figure 1.**

**Summary/Conclusions:** Our results highlight for the first time the independent prognostic significance of the light chain IGLV3-21 in CLL: the expression of an IGLV3-21 light chain confers a poor prognosis similar to UM patient irrespectively of concurrent expression of IGHV3-21 heavy chain or IGHV mutational status.
DURABILITY OF RESPONSES ON CONTINUOUS THERAPY AND FOLLOWING DRUG CESSATION IN DEEP RESPONDERS WITH VENETOCLOX AND RITUXIMAB


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Background: Venetoclax is a potent BCL-2 inhibitor that is approved as monotherapy for certain patients with relapsed or refractory chronic lymphocytic leukemia (CLL) in the United States, the European Union, and other countries.

Aims: Venetoclax combined with rituximab is being assessed in an ongoing Phase 1b study.

Methods: Minimal residual disease (MRD) was assessed in bone marrow using 24-color flow cytometry (minimum sensitivity: 0.01%). Patients who achieved complete remission (CR) or MRD-negativity could stop venetoclax and remain on study. Patients who manifested progressive disease while off therapy could re-initiate venetoclax and rituximab.

Results: Forty-nine patients, with a median of 2 (range: 1–5) prior regimens, were enrolled. As of July 2016, the overall response rate was 86%, the CR rate was 51%, and the bone marrow MRD-negativity rate was 57% (28/49) [Seymour et al. Lancet Oncol 2017]. The 24-month estimate for progression-free survival was 78.8% and that for duration of response was 87.8% (100% for patients with MRD-negative CR). Of the 28 patients attaining MRD-negativity, 22 achieved the status at 7 months, which was the first mandatory time point for assessment. The remaining six patients achieved MRD-negativity at the second assessment, which ranged from 12 to 22 months, since the timing of this test was not mandated. Twenty (41%) patients discontinued the study. Eleven had progressive disease while on therapy: five with Richter’s transformation between 1–9 months and six with CLL progression after a median of 26.4 months (range: 12–37). The other nine patients: withdrew consent (n=3), failed to report for follow-up evaluations (n=1), discontinued due to adverse events related to venetoclax (n=2; tumor lysis syndrome and worsening of peripheral neuropathy), or discontinued due to adverse events considered not related to therapy (n=3). Seventeen patients with MRD-negative CR, 2 MRD-positive CR, 5 MRD-negative PR, and 2 MRD-positive PR. Median duration of response on therapy is 27.9 months (range: 20.3–40.2). Sixteen patients discontinued venetoclax and remained on study as allowed per protocol following the achievement of a deep response (12 MRD-negative CR, 2 MRD-negative PR, 2 MRD-positive CR) (Figure 1). Their median time on venetoclax is 16.3 months (range: 5–38). Twelve of these patients remain in active follow-up and four discontinued without evidence of progression after achieving MRD-negative CR. Two patients with MRD-positive CR had increasing absolute lymphocyte count (ALC) and asymptomatic progression 24 months after stopping venetoclax, both with re-initiated venetoclax, 2 and 6 months after ALC >5x10⁹/L, and achieved partial remissions. The 10 patients with MRD-negativity in the bone marrow who remain in follow-up have a median duration of ongoing response off venetoclax of 13 months (range: 3–34).

Summary/Conclusions: Venetoclax with rituximab induces deep and durable responses, with 51% patients achieving CR and 57% achieving marrow MRD-negativity. Patients on continued therapy have durable responses. Additionally, responses are sustained at a median of 13 months among patients who achieve bone marrow MRD-negativity and elected per protocol to stop therapy, demonstrating that it is possible to discontinue venetoclax and maintain prolonged treatment-free remission. The 2 patients who progressed at 2 years off therapy responded to the reintroduction of venetoclax.

PREDICTIVE AND PROGNOSTIC IMPACT OF GENE MUTATIONS IN THE CONTEXT OF FLUDARABINE AND CYCLOPHOSPHAMIDE WITH OR WITHOUT OFATUMUMAB TREATMENT IN PATIENTS WITH REL/REF CLL


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Background: Recurrent mutations in genes such as TP53, SF3B1 and NOTCH1 are frequent in CLL and have in previous studies been associated with outcome. SF3B1mut, TP53mut, BIRC3mut and XPO1mut were adverse prognostic factors in patient cohorts with different therapies, and NOTCH1mut associated with poor outcome when rituximab was added to standard chemotherapy.

Methods: Baseline samples were available from 325 of 365 patients (89%) representative of the full analysis set of the clinical trial. Mutation analyses were performed via custom targeted Next Generation Sequencing (tNGS) for TP53, ATM, BIRC3, FBXW7, MYD88, EGR2 and XPO1 in the COMPLEMENT-2 trial (relapsed/refractory CLL, FC vs FC+ofatumumab (FCO), Robak et al., Leuk Lymphoma, 2017)

Results: In total we identified 365 mutations across the 9 genes in 202 of 325 patients (62.2%), with incidences of SF3B1mut 19.7%, TP53mut 18.8%, NOTCH1mut 16.3%, ATMmut 13.8%, XPO1mut 11.4%, BIRC3mut 4%, EGR2mut 3.1%, FBXW7mut 2.7% and MYD88mut 0.9%. We identified a variety of associations of mutational subgroups with genetic, clinical and laboratory parameters, such as TP53mut with del17p (p<0.01), NOTCH1mut, FBXW7mutand BIRC3mut with +12q (p<0.01, p=0.01 and p=0.05) and MYD88mut with del11q (p<0.01). XPO1mut and ATMmut associated with unmutated IGHV, CD79B expression on cell surface measured via flow cytometry was lower in ATMmut patients, whereas CD20 expression did not differ among the different mutational subgroups. TP53mut, EGR2mut and SF3B1mut patients had worse overall response to therapy (88% p<0.01, 50% p=0.02 and 72% p=0.05 respectively, vs 81% overall). Similar to the full analysis set, FC0 as compared to FC resulted in significant improved PFS (median 28.1 vs 18.8 months, HR=0.67, p<0.01), TP53mut and XPO1mutwere adverse prognostic factors for PFS (HR 1.93 p<0.01 and HR 1.85, p<0.01 respectively), but only TP53mut for decreased OS (HR 2.11 p<0.01). All other mutations, in particular SF3B1mut and NOTCH1mut, did not significantly impact PFS or OS. To identify factors of independent clinical
impact, we performed multivariable Cox regressions for PFS and OS including treatment, IGHV status and all cytogenetic and mutational subgroups. For PFS, the following independent prognostic factors were identified: FCO therapy (HR 0.64 p<0.01), del17p (HR 5.08 p<0.01), unmutated IGHV (HR 2.0 p=0.01), TP53mut (HR 1.75 p<0.01) and XPO1mut(1.86 p<0.01). Del17p (HR 4.79 p<0.01), unmutated IGHV (HR 1.69 p=0.04) and TP53mut (HR 1.76 p=0.03) were identified as independent prognostic factors for OS. With focus on the predictive value of gene mutations, we found a beneficial effect of the addition of ofatumumab to chemotherapy irrespective of TP53 mutation (HR 0.52 p=0.02 for TP53mut and HR 0.68, p=0.02 for TP53mt). Regarding NOTCH1, ofatumumab 4x was only beneficial in NOTCH1mt but not in NOTCH1mt patients (HR 0.64, p<0.01 and HR 0.86, p=0.05) (Figure 1).

Summary/Conclusions: In the COMPLEMENT-2 trial evaluating FCO against GC in relapsed/refractory CLL patients, we found TP53mut and XPO1mut but not SF3B1mut or NOTCH1mut as independent prognostic factors for PFS. Notably, a benefit of ofatumumab addition to FCO treatment was observed among NOTCH1mt but not among NOTCH1mt patients indicating NOTCH1 mutation status as a predictive marker in the context of type-1 CD20 antibody addition to chemotherapy.

P249

RESULTS OF A PHASE II MULTICENTER STUDY OF OBINUTUZUMAB PLUS BENDAMUSTINE IN PTS WITH PREVIOUSLY UNRETIRED CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Bendamustine (B) plus rituximab (R; BR) is a commonly used front-line (1L) treatment for chronic lymphocytic leukemia (CLL). The CLL10 study reported an overall response rate (ORR) of 96% and complete response (CR) rate of 31% with BR. Obinutuzumab (GA101; G) is a glycoengineered, type II anti-CD20 monoclonal antibody. A randomized Phase III trial in 1L CLL pts showed that G significantly improved progression-free survival (PFS) and CR rate compared with R, when used in combination with chlorambucil (Goede 2014). B plus G (BG) was evaluated in a subgroup of CLL pts in the GREEN study (Stilgenbauer 2015).

Aims: The aim of this Phase II study (NCT02320487) is to evaluate the efficacy and safety of BG as 1L treatment for CLL pts.

Methods: 102 pts with previously untreated CLL received BG, consisting of 6 cycles of G (cycle [C] 1: 100 mg/day (D) 1, 900mg D2, 1000mg D8 and D15; C2– 6: 1000mg D1 and B (80mg/m²; C1, D2 and C3; C2–6, D1 and D2). Each cycle was 28 days. The primary endpoint was CR assessed using IWCLL criteria. Secondary endpoints included ORR, PFS, overall survival, and minimal residual disease (MRD). Median follow-up at the time of analysis was 11.0 months.

Results: Median pt age was 61 yrs (range 35–90); 68.6% were male; 44.1% had Rai stage 3–4. For evaluated pts, IgVH status was 32.9% mutated and 67.1% unmutated. Incidences of trisomies 12, normal cytogenetics, and deletions of 13q, 11q, and 17p were 23.4%, 37.5%, 17.2%, 15.6%, and 6.3%, respectively. Investigator-assessed CR rate was 49.0% (95% CI 39.0–59.1) and ORR was 89.2% (95% CI 81.5–94.5) after 6 cycles. MRD negativity in blood, as measured by next-generation sequencing analyses, was achieved in 42.7% of pts at the end of induction response assessment and in 75.5% of pts at any time following treatment. MRD negativity in bone marrow (BM) was 60.8% in pts with BM samples. The most common adverse events (all grades [Gr]) were infusion reactions (72.5%), nausea (52.0%), pyrexia (36.3%), neutropenia (34.3%), fatigue (34.3%), constipation (26.5%), and rash (26.5%). The most common Gr 3–4 adverse event was neutropenia (26.5%). Incidence of Gr 3–4 infections was 11.8%. Incidence of tumor lysis syndrome was 4.9% (all Gr 3). Three pts died; none were deemed related to study treatment or CLL by investigators.

Summary/Conclusions: BG is an effective regimen for 1L treatment of CLL pts inducing a high CR rate after 6 cycles of therapy. No unexpected safety signals were observed.

P250

RELATIVE SURVIVAL REACHES A PLATEAU IN HAIRY CELL LEUKEMIA: A POPULATION-BASED STUDY ON INCIDENCE, PRIMARY TREATMENT AND SURVIVAL AMONG 1,427 PATIENTS DIAGNOSED IN THE NETHERLANDS, 1989-2014

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Background: The introduction of cladribine and pentostatin has revolutionized the management of HCL as from the late 80s. As a result of that evolution, HCL patients (pts) are rarely included in clinical trials. Population-based studies can inform on issues related to outcomes of HCL pts managed in daily practice. At present, however, population-based studies that assess patterns of incidence, treatment and survival in HCL are very scarce.

Aims: The aim of this comprehensive nationwide population-based study was to assess trends in incidence, primary treatment and survival among HCL pts diagnosed in the Netherlands.

Methods: We selected all adult (≥18 years) pts diagnosed with classic HCL in the Netherlands between 1989-2014 from the national Netherlands Cancer Registry with survival follow-up through February, 2016. Age-standardized incidence rates (ASR) were calculated per 100,000 person-years and standardized according to the European standard population. Data on primary treatment (i.e. no therapy, chemotherapy [CT] and immunotherapy [IT]) were available for individual pts. Pts were categorized in 2 periods (1989-2000 and 2001-2014) and 3 age groups (18-59, 60-69 and ≥70 years). We calculated relative survival (RS) and the relative excess risk of mortality as measures of disease-specific survival.

Results: We included a total of 1,427 newly diagnosed HCL pts in the study (median age, 59 yrs; age range, 22-95 years; 77% males). The annual ASR of HCL remained quite stable over time and was 3.1 and 3.3 in the first and last period, respectively. Men had a higher overall incidence than women (5.3 v 1.3 in 2000-2014). The age-specific incidence rates for males were 5.5, 15.0 and 15.3 in 2001-2014 for the three age groups. The corresponding rates for females were 1.2, 3.1 and 5.5. The application of CT increased over time for all age groups. The proportions of CT for the three age groups were 56, 51 and 34% in 1989-2000, as compared with 81, 73 and 53% in 2001-2014. The corresponding proportions for IT were 21, 13 and 17% in 1989-2000, as compared with 2, 1 and 4% in 2001-2014. Lastly, the corresponding proportions for pts who did not receive therapy were 23, 36 and 49% in 1989-2000, as compared with 17, 26 and 42% in 2001-2014. Overall, when corrected for age and sex, pts diagnosed in 2001-2014 had 49% lower excess mortality during the first 10 years after HCL diagnosis, as compared with pts diagnosed in 1989-2000 (P<.005). Ten-year RS (95% confidence intervals) was impressive for pts age 18–59, namely 92% (88% - 96%) and 98% (94% - 100%; P=.176) in the first and last period, respectively (Figure 1a). Most of the significant improvement was observed in pts age ≥60. More specifically, 10-year RS for pts age 60-69 increased from 82% (71% - 92%) to 99% (89% - 100%; P=.009; Figure 1b), and for pts age ≥70 from 67% (49% - 86%) to 89% (86% - 92%; P=.02; Figure 1c) between the first and last periods. In addition, older age (P<.001), but not sex (P=.058), was associated with higher excess mortality.

Figure 1.

Summary/Conclusions: The incidence of HCL remained stable during a 26-year period in the Netherlands. RS for pts diagnosed in the period 2001-2014 eventually reached a plateau, indicating that by then their survival is comparable to that of the general population. Survival was already excellent for younger patients throughout the entire study period. Survival improvement was most pronounced for pts aged ≥60. This could be explained by the increased use CT over time. Population-based cancer registries are useful instruments to assess outcomes of pts rarely included in clinical trials.
CUMULATIVE ILLNESS RATING SCALE PROVIDES PROGNOSTIC INFORMATION BEYOND THE INTERNATIONAL PROGNOSTIC INDEX FOR CHRONIC LYMPHOCYTIC LEUKEMIA: AN ACROSS-TRIAL ANALYSIS BY THE GCLLSG

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Background: CLL-IPI is a prognostication tool to stratify patients with chronic lymphocytic leukemia (CLL) for low, intermediate, high, or very high risk. CLL-IPI uses age, Binet stage, beta-2-microglobulin, TP53 mutation, and IGHV mutational status, but not comorbidity as weighted factors to model prognosis. CIRS is a tool which allows assessing and quantifying burden of comorbidity in individual patients.

Aims: To validate CIRS in CLL and to assess whether CIRS is of further value when estimating prognosis by CLL-IPI in CLL.

Methods: This is a comprehensive evaluation of CIRS in 2158 patients pooled from the CLL8, CLL10, and CLL11 trials of the German CLL Study Group (GCLLSG). Median observation time was 55 months. All patients had CIRS data prospectively assessed prior to study treatment (689 FCR, 409 FC, 279 BR, 333 GCLB, 330 RCLB, 118 CLB).

Results: Median age was 64 years; 69% of patients were males, and 50% had ECOC performance score of 1 or higher. Complete information on age, Binet stage, beta-2-microglobulin, TP53 deletion and/or TP53 mutation, and IGHV mutational status was available in 1761 of the 2158 patients. Distribution of CLL-IPI risk groups was as follows: 275 (16%) low risk, 653 (37%) intermediate risk, 712 (40%) high risk, 121 (7%) very high risk. CLL-IPI uses age. Binet stage, beta-2-microglobulin, 17p deletion / TP53 mutation, IGHV mutational status, but not comorbidity as weighted factors to model prognosis. CIRS is a tool which allows assessing and quantifying burden of comorbidity in individual patients.

CIRS score was associated with higher risk of grade 3/4 adverse events as well as premature treatment discontinuation during or after treatment with FCR / FC / BR but not GCLB / RCLB / CLB.

Summary/Conclusions: Findings suggest that CIRS provides prognostic information beyond the CLL-IPI and can be used as a comorbidity assessment (e.g. by CIRS) in addition to the CLL-IPI therefore appears reasonable when estimating overall prognosis and deciding treatment in CLL.
**P253**

**FINAL RESULTS OF THE PHASE IB GAlTON TRIAl IN CHRONIC LYMPHOCYTIC LEUKEMIA: DURABLE REMISSIONS WITH FRONTLINE OBINUTUZUMAB (G) PLUS FLUARABINE/CYCLOPHOSPHAMIDE (G-FC) OR BENDAMUSTINE (G-B)**

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**Background:** GALTON was an open-label, parallel-arm, non-randomized, multicenter, Phase Ib study (NCT01300247) investigating safety and preliminary efficacy of G-FC or G-B in previously untreated CLL.

**Aims:** We report final results for the planned 36-months’ (mo) follow-up (35/41 pts; median observation 40.4 [17.6–43.6] mo); initial results were reported previously (Brown et al. 2015).

**Methods:** Eligible pts met iwCLL 2008 criteria for therapy, were considered fit for chemoimmunotherapy by the investigator, and provided informed consent. Each center selected treatment (G-FC or G-B) for their pts. G was administered intravenously (IV; 100mg day [D] 1, 900mg D2, 1000mg D8 and 15 cycle [C] 1: 1000mg D1 C2–6) with FC (fluorabrine 25mg/m2 IV and cyclophosphamide 250mg/m2 IV D2–4 C1, D1–3 C2–6 or B (90mg/m2 IV D2–3 C1, D1–2 C2–6). Each cycle was 28 days. The primary endpoint was safety and tolerability of G-chemotherapy.

**Results:** 21 pts were enrolled in the G-FC arm and 20 in the G-B arm. Median age was 60 (25–80) years, 78% of pts were male, and around one-third had Rai stage III/IV disease. Median time from diagnosis to therapy was 24 mo (G-FC: 18 mo; G-B: 9 mo). At last follow-up, 37 pts were alive in follow-up: G-FC (n=18: 2 lost to follow-up) and G-B (n=19: 1 event of progressive disease—PD—occurred in each arm, and 1 pt per arm died due to an adverse event (AE; G-B: respiratory failure; G-FC: unknown in the setting of unresolved Grade (Gr) 4 pancytopenia); neither was considered treatment related. Due to the small number of events, median PFS and OS could not be estimated; however, 3-year OS was 95% for each arm (95% CI G-FC, 68–99; G-B, 70–99). Post-treatment, 10/41 pts (24.4%) experienced ≥1 Gr3–5 AE: 2/21 pts (9.5%) in the G-FC arm and 8/20 pts (40.0%) in the G-B arm. 7 serious AEs were reported in 4 pts, all in the G-B arm: these included pneumonitis and respiratory failure (as noted above), Gr4 leukaemia/neutropenia, small cell lung cancer and Gr4 pneumo-thorax, and melanoma. During follow-up, 6 pts had ≥1 Gr3–4 AE of neutropenia, including 4/20 pts (20.0%) in the G-B arm and 2/21 pts (9.5%) in the G-FC arm. At end of treatment, all pts were B-cell depleted (B-cell count < 0.07x10^9/L). Within 6–12 mo of follow-up, very few pts had recovered B-cell depletion (G-FC: 2/19 pts [10.5%]; G-B: 0/20 pts). At 36 mo follow-up, 9/19 pts (47.3%) in the G-FC arm had recovered, 1/19 (15.8%) were still depleted, and 7/19 did not have data available. In the G-B arm, 6/20 pts (30%) had recovered, 1 was still depleted, and 13/20 had no available data. In a single center exploratory analysis, 9 pts (G-FC) underwent 4-color flow cytometry testing of peripheral blood for minimal residual disease (MRD) 8–14 mo after therapy; all were negative. 8 of these pts (G-FC) who were MRD-negative by 4-color flow cytometry were also tested with the ClonoSEQ immunoglobulin sequencing assay: 4 were MRD-positive and 4 MRD-negative. 4 pts who were MRD-negative on both assays remain in remission, while 2/4 pts who were positive by ClonoSEQ died after follow-up, one of Richter’s transformation complicated by pneumonia and the other related to MDS. Another pt who was MRD positive by ClonoSEQ underwent allogeneic stem cell transplantation and remains in remission.

**Summary/Conclusions:** We conclude that G plus either FC or B results in excellent long-term disease control in previously untreated pts with CLL, and has comparable side-effects to other chemo-immunotherapies reported.

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**P254**

**THE PROGNOSTIC SIGNIFICANCE OF CLl-IPI AFTER REDUCED INTENSITY CONDITIONING ALLOGENEIC STEM CELL TRANSPLANTATION IN CHRONIC LYMPHOCYTIC LEUKEMIA: THE MAYO CLINIC EXPERIENCE**

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**Background:** Allogeneic stem cell transplant (SCT) remains the only potentially curative option for chronic lymphocytic leukemia (CLL) patients. However, up to 40% of patients treated with Reduced Intensity Conditioning (RIC) - SCT relapse after transplantation. Recently the CLL International Prognostic Index (CLL-IPI) was validated as a predictor of 5 year overall survival in CLL patients.

**Aims:** In this analysis, we aimed to elucidate the factors that may predict the outcomes following RIC SCT, including the CLL-IPI.

**Methods:** This is a retrospective analysis of all CLL patients who underwent RIC-SCT at Mayo Clinic between 2006-2013. The study was approved by the Institutional Review Board. The prognostic value of several CLL, patient and transplant related variables were analyzed. Continuous variables were reported as mean and compared using the T-test. Dichotomous outcomes were compared using the chi-square test. Survival was estimated and compared using the Kaplan Meier and Log Rank tests.

**Results:** Between 2006 and 2013, 50 patients with a median age of 56 years old underwent RIC-SCT for the treatment of CLL. The median time from diagnosis to RIC-SCT was 4.7 (0.6-22.9) years. Fourteen (28%) patients had 17p deletion at time of transplantation. CLL-IPI prognostic score calculated prior to transplant was intermediate in 30%, high in 42% and very high in 28% of patients. Disease status at the time of transplant was partial or complete remission in the majority of patients (39 patients, 78%). The overall transplant related mortality (TRM) was 6% and the 5-year non-relapse mortality was 14%. Relapse rates at 5 years were 54%. Acute graft versus host disease (GVHD) developed in 30 (60%) of patients and chronic GVHD was noted in 32 patients (64%). We evaluated the impact of CLL characteristics, disease status, and patient and transplant characteristics on clinical outcomes. Development of chronic GVHD post-transplant was the dominant predictor of both disease-free survival (DFS) (HR 0.29, 95% CI=0.10-0.69, P=0.006) and OS (HR 0.04, 95% CI=0.01-0.19, P<0.0001, Figure 1A). Very high CLL-IPI risk category (28% of patients) was associated with high relapse rates (82%) post RIC-SCT. DFS was also different between different CLL-IPI categories (18.2% in very high 52.9% in high vs 66.7% in intermediate, p=0.04, Figure 1B). However, there was no significant difference in overall survival suggesting potential benefits from novel therapies in relapsed patients. Given that development of chronic GVHD was the most significant predictor for OS, we evaluated what pre-treatment characteristics were associated with chronic GVHD, and transplant characteristics that were associated with subsequent development of chronic GVHD. ZAP70 over expression (OR 0.09 [95% CI 0.01-0.79], p=0.03), disease status at transplant (progression versus remission OR 0.22 [95% CI 0.05-0.92], p=0.038), and alemtuzumab exposure within 3 months of transplantation were associated with lower rates of chronic GVHD (OR 0.08 [95% CI 0.01-0.79], p=0.03). CLL-IPI was not a significant predictor for the development of chronic GVHD in our analysis.

**Summary/Conclusions:** This study found that the development of chronic GVHD post-transplant is themost significant predictor for both OS and DFS in surviving patients after RIC-SCT in CLL. Interestingly, 82% of patients with very high risk CLL-IPI relapsed after RIC-SCT. This is the first report to evaluate the prognostic significance of CLL-IPI for stratifying post-transplant outcomes and to identify high relapse rates in the very high risk CLL-IPI category.
P255

IMPACT OF ABCG2, OCT1 AND ABCB1 (MDR1) ON TREATMENT FREE REMISSION IN AN EUROSKI SUBTRIAL


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Background: Several studies showed that tyrosine kinase inhibitors (TKIs) can safely be discontinued in patients with sustained deep molecular response. So far, deep molecular response (DMR) and treatment duration were predictive for successful treatment-free remission (TFR) whereas age, risk score, gender and molecular response level before stopping were without influence (Mahon FX, et al. ASH 2016). In addition, biomarkers like NK-cells and CD8+ cells (lander M. et al. and Schütz C. et al., Leukemia 2017) seem to be of impact. ABCG2, OCT1 and ABCB1 are known to play a crucial role in acquired pharmacokinetic drug resistance and DMR in the context of nilotinib, imatinib and dasatinib. The influence of these mechanisms have not yet been analyzed for their correlation with TFR.

Aims: The human leukocyte antigen-G (HLA-G) gene encodes a tolerogenic protein known to promote tumor immune-escape mechanisms. Aims: We investigated the potential role of HLA-G polymorphisms and soluble HLA-G molecules in susceptibility to chronic myeloid leukemia (CML), as well as in achievement and maintenance of deep molecular remission (MR4.5) in 68 patients treated with tyrosine kinase inhibitors (TKIs).

Methods: The entire HLA-G gene was amplified by long-range PCR and sequenced using next-generation sequencing (NGS) with Illumina’s Nextera® technology and a 300 bp paired-end read protocol. The BioVendor sHLA-G ELISA (RD194070101HR sHLA-G ELISA - EXBIO Praha a.s. Biovendor) immunocassay was used for the quantitative measurement of HLA-G1 and HLA-G5 soluble forms in EDTA-plasma samples

Results: The frequency of the G*01:03 allele was significantly associated to G01:01:03 (109.2±39.5 vs 44.46; p=0.001). Patients carrying the G01:01:02 allele had a significantly higher mean value of soluble HLA-G compared to patients carrying G01:01:03 (109.2±39.5 vs 39.9±8.8 units/ml; p=0.03), and showed significantly lower EFS compared to patients with other allelic combinations (62.3% vs 90.0%; p=0.05). Moreover patients carrying the G01:01:03 allele had significantly higher rates of MR4.5 (100% vs 85%), with earlier achievement of deep MR4.5 (median of 8 vs 58 months, p=0.001). TKIs were discontinued in 24 patients after 2 years of confirmed MR4.5. Treatment free remission (TFR) was 57.7%. None of the patients homozygous for the G01:01:01 or G*01:01:02 allele remained in TFR (0% vs 68.4%, p=0.023) (Figure 1). All patients carrying the G*01:01:03 allele remained in TFR.

Figure 1. Summary/Conclusions: HLA-G alleles with higher secretion of soluble HLA-G...
G would seem to be associated with lower EFS and TFR, possibly because of a stronger inhibitory effect on the immune system in favor of tumor escape mechanisms. Conversely, the allele associated to lower levels of sHLA-G promoted achievement of MR4.5 and TFR, suggesting increased cooperation of the host immune system in CML cell clearance.

P257

DURABLE TREATMENT-FREE REMISSION AFTER STOPPING SECOND-LINE NILOTINIB IN PATIENTS WITH CHRONIC MYELOID LEUKAEMIA IN CHRONIC PHASE: ENESTOP 96-WK UPDATE


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Background: ENESTop (NCT01698905) is evaluating the ability to stop treatment and remain in TFR in pts with CML-CP who achieved a sustained deep molecular response (MR) after switching from imatinib (IM) to NIL. In the primary analysis, 57.3% of pts (73/126) who stopped treatment remained in TFR (no loss of major MR, BCR-ABL1<0.1% on the International Scale (IS), no confirmed loss of MR4 [BCR-ABL1 ≤0.01%], and no treatment reinitiation) at 48 wk.

Aims: To evaluate the proportion of pts remaining in TFR at 96 wk after stopping second-line NIL in ENEStop.

Methods: Eligible pts had ≥3 y of prior tyrosine kinase inhibitor treatment (>4 wk IM, then ≥2 y NIL) and achieved MR4.5 (BCR-ABL1 ≤0.0032%) after switching to NIL. All pts provided informed consent. Enrolled pts continued NIL for 1 y in the consolidation phase (MR assessed every 12 wk). Pts without confirmed loss of MR4.5 during consolidation were eligible to enter the TFR phase (MR4.5 assessed at 48 wk; in the first 48 wk for the first 27 pts and then every 12 wk). Pts with loss of MMR or confirmed loss of MR4 reinitiated NIL. This analysis was conducted when all pts who entered the TFR phase had completed 96 wk of TFR, reinitiated treatment, or discontinued from the study (data cutoff, 7 Nov 2016).

Results: In the consolidation phase, 67 of the 126 pts (53.2% [95% CI, 44.1% - 62.1%]) who entered the TFR phase remained in TFR. Four pts who were in TFR at 48 wk reinitiated NIL due to confirmed loss of MR4 at 60, 72, 90, and 96 wk, respectively. Two other pts discontinued from the study between 48 and 96 wk due to pregnancy (last BCR-ABL1 of 0.0035% at 60 wk) and pt decision (maintained MR4.5 through 90 wk), respectively. Based on Kaplan-Meier analysis, the median duration of treatment-free survival has not been reached and the curve appeared to plateau (Figure 1). Of 56 pts who reinitiated NIL by the data cutoff, 52 (92.9%) regained MR4 and MR4.5, and the time by which 50% of pts regained MR4.5 was shorter for pts who reinitiated NIL compared to baseline (27.3±5.7 mg/l). In the patient cohort, IMA resulted in a significant increase in adiponectin levels compared to baseline (27.3±5.7 mg/l). In contrast, second line NIL showed a significant decrease in the second 48 wk of TFR vs the first 48 wk. Overall, these results demonstrate the durability of TFR after stopping NIL in pts who achieved a sustained deep MR after switching from IM to NIL.

Figure 1.

P258

NILOTINIB-INDUCED METABOLIC DYSFUNCTION: INSIGHTS FROM A TRANSLATIONAL PILOT STUDY USING IN VITRO ADIPOCYTE MODELS AND PATIENT COHORTS

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Background: Impaired glucose and lipid metabolism is an adverse effect associated with nilotinib (NILO), a tyrosine kinase inhibitor (TKI) used in the treatment of chronic myeloid leukaemia (CML). Indeed the 5-year safety analysis of the ENEStnd trial observed elevations in blood glucose and lipid levels in the NILO arms; importantly NILO-treated patients also showed an increased incidence of arterial occlusive events. Adipose tissue is a key regulator of lipid and glucose homeostasis; dysregulation of adipogenesis, altered adipocyte lipid accumulation and reduced insulin sensitivity are implicated in the pathogenesis of metabolic disease. We investigated the effect of NILO on adipose tissue to explain the mechanisms behind NILO-associated metabolic adverse effects.

Aims: i) To study the effect of NILO and imatinib (IMA) on adipocyte function and adipokine secretion using an in vitro adipocyte model; ii) To utilise the in vitro model to explore potential therapeutic strategies to reverse NILO-mediated effects, and iii) To validate the in vitro results in a pilot patient cohort.

Methods: Differentiating 3T3-F442A mouse adipocytes were incubated with clinically relevant concentrations of NILO (1-10µM) and IMA (5µM), in the presence or absence of telmisartan (1-10µM), an angiotensin receptor blocker with potential beneficial effects on insulin sensitivity and lipid homeostasis. Cyto-toxicity and adipogenesis were assessed by MTT assay and Oil Red O staining, respectively. Expression of adipogenic genes, peroxisome proliferator-activated receptor gamma (PPARγ), lipin1 (LPIN1), sterol regulatory element-binding protein 1 (SREBP1) and glucose transporter 4 (GLUT4) were investigated by quantitative PCR and secreted adiponectin was measured by ELISA. Plasma samples were collected from 30 CML patients on either NILO (first line, n=8; 48 patients total) or IMA (first line, n=15) at baseline and at 3 and 12 months of therapy, and adiponectin was measured by ELISA. Data are presented as mean ± SD for 20µM incubations but full concentration response relationships were measured.

Results: Neither NILO nor IMA were cytotoxic to the adipocytes at clinically relevant concentrations. A dose dependent reduction in lipid accumulation was observed for NILO (for 20µM, 0.76 ± 0.005 absorbance units; p<0.01) but not IMA (0.98 ± 0.007), compared to vehicle control. NILO, but not IMA, dose dependently downregulated the mRNA expression of PPARγ (52% downregulation), LPIN1 (28% downregulation) and SREBP1 (54% downregulation). Both NILO and IMA resulted in significant downregulation of GLUT4 mRNA (NILO, 93%; IMA, 79%; p<0.01) and of secreted adiponectin (NILO, 5.99ng/ml; IMA, 31ng/ml; both p<0.01 in comparison to vehicle control, 79.2ng/ml). Co-incubation with telmisartan resulted in significant reversal of NILO-mediated effects on lipid accumulation, adipogenic gene expression and adiponectin secretion. In the patient cohort, IMA resulted in a significant increase in adiponecin levels at 3 (38.4±3.7mg/l; p<0.01) and 12 (36.7±7.2mg/l; p<0.01) month time points compared to baseline (27.3±5.7mg/l). In contrast, second line NILO showed a trend for reduction in adiponectin at both 3 (15.2±1.8mg/l; p=NS) and 12 months.
EARLY PREDICTION OF THE MOLECULAR RESPONSE TO BCR-ABL1 TYROSYNE KINASE INHIBITORS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

Summary/Conclusions: A BCR-ABL1 transcript level measured at 1 month after the initiation of a TKI may be used as an early indicator to reliably predict the MMR achievement by 12 months in patients with CP-CML. The level obtained at 3 months appears to accurately predict the MMR. Further studies are needed to evaluate the association between the transcript level at 1 month and long-term clinical outcomes.

P259

EARLY PREDICTION OF THE MOLECULAR RESPONSE TO BCR-ABL1 TYROSYNE KINASE INHIBITORS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

Summary/Conclusions: NILO-induced detrimental effects on adipoocyte lipid accumulation and adiponectin secretion could be the mechanistic basis for NILO-mediated metabolic dysfunction. This was reversed by telmisartan, a PPARγ partial agonist. A larger sample size is required to fully characterise the effect of TKIs on metabolic parameters in the patient population.

P260

Abstract withdrawn.

P261

A HIGH SENSITIVITY HIGH SPECIFICITY DIGITAL PCR ASSAY FOR BCR-ABL

Summary/Conclusions: A BCR-ABL1 transcript level measured at 1 month after the initiation of a TKI may be used as an early indicator to reliably predict the MMR achievement by 12 months in patients with CP-CML. The level obtained at 3 months appears to accurately predict the MMR. Further studies are needed to evaluate the association between the transcript level at 1 month and long-term clinical outcomes.

P260

Abstract withdrawn.

P261

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Summary/Conclusions: A BCR-ABL1 transcript level measured at 1 month after the initiation of a TKI may be used as an early indicator to reliably predict the MMR achievement by 12 months in patients with CP-CML. The level obtained at 3 months appears to accurately predict the MMR. Further studies are needed to evaluate the association between the transcript level at 1 month and long-term clinical outcomes.
Background: A second-generation tyrosine kinase inhibitor (TKI), dasatinib, is more potent in inhibiting BCR-ABL kinase activity than imatinib. We had previously reported an interim analysis of 63 patients with CML-CP who had discontinued dasatinib treatment after maintaining a deep molecular response (DMR) for more than a year (Lancet Haematol, 2015; 2 (12):e528-35) and demonstrated that dasatinib could be safely discontinued in patients with a DMR of at least 12 months. Longer follow-up results would be more critical in the treatment of CML.

Aims: In this trial, the total follow-up duration was set as 36 months after the discontinuation. The aim of the current follow-up study was to investigate whether those patients were able to discontinue dasatinib treatment for a longer follow-up period without relapse.

Methods: The eligibility criteria for pre-registration included CML-CP patients, 15 years or older, receiving dasatinib treatment as the second-line or subsequent therapy after imatinib. All participants gave written informed consent. In this trial, DMR was defined as "no detectable BCR-ABL1 transcript determined using the international scale-based RQ-PCR at a single central laboratory (BML Inc., Tokyo; the cutoff corresponded to BCR-ABL1 0.0069% IS or molecular response (MR) 4.0)." Patients who showed a sustained DMR for 1 year (1-year consolidation phase) were subsequently included in the dasatinib-discontinuation stage. RQ-PCR was performed monthly for the first 12 months, and then every 3 months for the second year, and every 6 months for the third year, after discontinuing dasatinib. Relapse was defined as any positivity of BCR-ABL1 transcript by RQ-PCR even at one analysis point. In the present study, we assessed the estimated overall treatment-free remission (TFR) after discontinuing dasatinib with 3 years of follow-up. In addition, we also evaluated the impact of immunological profiles, including the cell counts of T and NK cell subsets in the peripheral blood throughout the 1-year consolidation phase, on TFR.

Results: Sixty-three patients were included in the dasatinib-discontinuation stage. The estimated overall treatment-free remission (TFR) after discontinuing dasatinib was 44.4% (95% confidence interval [CI], 32.0-56.2) at 36 months. A high count of NK-cell phenotypes (CD3-CD56+ cells ≥539 cells/μl and CD16+CD56+ cells ≥506 cells/μl) and a low count of gd+ T-cells (<120 cells/μl) were detected to be significant factors affecting molecular relapse in the interim analysis; these showed sustained significance as predictors of a favorable TFR (P=0.0475, 0.0202, and 0.0093, respectively).

Summary/Conclusions: As the overall provability of TFR was relatively stable even for a longer follow-up period, our findings provided more compelling evidence supporting dasatinib discontinuation after a DMR for more than 1 year; this is feasible especially in patients with imatinib intolerance. We also confirmed that the counts of NK cells and functionally specific T-cells in the peripheral blood during dasatinib treatment might affect the TFR following dasatinib discontinuation.
Hematopoiesis, stem cells and microenvironment

**P264**

**ACUTE MYELOID LEUKEMIA ALTERS THE PERMEABILITY OF THE BONE MARROW VASCULAR MICROENVIRONMENT, FOSTERING DISEASE PROGRESSION AND DRUG RESISTANCE**

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**Background:** The biological and clinical behavior of hematological malignancies is not only determined by the properties of the leukemic cells themselves, but it is also highly affected by the interaction with the microenvironment, pointing to the existence of an active crosstalk between the two compartments. Previous studies showed that acute myeloid leukemia (AML) actively modify endothelial cells in vivo via several pathways, mainly mediated by VEGF. However, anti-VEGF therapies haven’t produced successful results in clinical trials.

**Aims:** Our aim is to perform an extensive study of the vascular niche in the bone marrow (BM) of AML xenografts to provide a global picture of the vasculature in AML disease and design new therapeutic strategies.

**Methods:** We combined the use of mouse models of AML, human AML derived xenografts (PDx) and direct analysis of patients derived samples to study the vascular niche in AML disease. We used two-photon confocal microscopy as a powerful tool to functionally image the BM vasculature in vivo. We used RNA-sequencing to study the AML-associated transcriptomic profile in vascular endothelial cells.

**Results:** We found several abnormalities in the vascular architecture and function in PDx, such as increased number of endothelial cells, increased microvascular density (MVD), loss of normal sinusoidal architecture and increased hypoxia. Moreover, vascular permeability was increased as measured via two-photon imaging. Interestingly, induction chemotherapy failed to normalize the vascular permeability in the BM, although it significantly reduced the AML engraftment. Via high-throughput transcriptomic analysis, we showed that AML-induced hypoxic environment altered the molecular signature of vascular endothelial cells, activating pro-angiogenic pathways and positively regulating the response to hypoxia. We identified increased nitric oxide (NO) as a major mediator of the AML-induced vascular leakiness in the BM. Notably, increased NO levels were found also in BM aspirates of patients at diagnosis compared to healthy donors, and failure in reducing NO levels after chemotherapy was associated with a higher incidence of unsuccessful treatment. Strikingly, inhibition of NO production in mouse models of AML and in AML-derived PDx reduced vascular permeability, preserved normal HSC function and significantly improved treatment response (Figure 1).

**Figure 1.**

**Summary/Conclusions:** We have shown an altered highly permeable vascular niche in the BM of AML PDx, mainly caused by increased NO production by the endothelial niche, contributing to disease progression and treatment failure. Our data call for clinical trials incorporating NOS inhibitors during the remission phase, to target the abnormal vascular niche and improve AML treatment response.

**P265**

**BUILDING HUMAN BONE MARROW-LIKE MODELS TO STUDY NICHE INTERACTIONS**

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**Background:** Previously, we have reported that our human bone marrow-like scaffold (huBM-sc) xenograft model allows the engraftment and outgrowth of normal and malignant hematopoiesis (e.g. multiple myeloma (MM) and acute lymphoblastic leukemia (ALL) (Groen et al. Blood 2012; Gutierrez et al. JCI 2014) and more recently acute myeloid leukemia (AML; Antonelli et al. Blood 2016). These studies show that i) engraftment is not correlated with prognostic risk-groups, ii) there is preferential outgrowth in humanized scaffolds compared to the murine BM, iii) the huBM-sc environment results in better maintenance of self-renewal potential and less clonal drift of the leukemic cells. Although the presence of human osteoblasts and bone microms a human BM niche more closely than the murine BM in standard xenotransplant models (e.g. NOD-SCID/NSG mice), still some essential components of the human BM niche, i.e. human bone vessels, are missing.

**Aims:** To implement human vasculature in the huBM-sc xenograft model in order to create a multi-tissue compartment that “maximally humanizes” the BM-like niche of our scaffolds.

**Methods:** Towards successful implementation of a human vascular system scaffold material composition (biphasic calcium phosphate vs tricalcium phosphate (TCP)); ii) scaffold shape (particles vs tubes); iii) different types of matrigel for cord blood-derived endothelial progenitor cells (CB-EPCs) embedding.

**Results:** Histological analysis of these fully humanized scaffolds showed a large homogeneous vascular network of human bone vessels, preserving normal HSC function and significantly improved treatment response. Thus, with the addition of human CB-EPCs and BM stromal cells, our scaffold systems now simulate both endothelial and vascular niches of the BM, thereby more closely recapitulating the human hematopoietic microenvironment.

**P266**

**MULTISCALE IMAGE-BASED QUANTITATIVE ANALYSIS OF BONE MARROW Stromal NETWORK TOPOLOGY REVEALS STRICT SPATIAL CONSTRAINTS FOR HEMATOPOIETIC-STROMAL CELLULAR INTERACTIONS**

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**Background:** Adult bone marrow (BM) cavities host continuous, demand adapted and high throughput blood cell production, which is maintained by a rare population of self-renewing, multipotent hematopoietic stem cells (HSCs). Aside from its diverse hematopoietic content, the BM is populated by a heterogeneous fraction of mesenchymal, endothelial and neural stromal cells, which provide the necessary tissue infrastructure for hematopoiesis by playing fundamental regulatory roles in hematopoietic development. Recent evidence suggests that tissue regions around BM venous microvesse (termed sinuses), which are enriched for mesenchymal CXCL12-abundant reticular cells (CARs), serve as the principal regulatory niches for HSCs as well as other hematopoietic progenitor populations. Despite this proposed role as putative specific niche-restricted components, comprehensive data on the frequency, global spatial distribution and topology of sinusoidal endothelial and CAR cell networks is largely lacking to date.

**Aims:** The principal aim of our work is to employ state of the art imaging techniques to perform a detailed 3D quantitative and structural analysis of the BM stromal infrastructure, with a special focus on sinusoidal microvasculature and the CAR cell mesenchymal component, both of which are essential regulators of HSC maintenance.
**P267**

**TEMPLATED V(D)J INSERTIONS ARE A NOVEL BIOLOGIC MECHANISM FOR B-CELL RECEPTOR REPERTOIRE DIVERSIFICATION**

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**Background:** Recently, LAIR1 insertions at the V-D junction were described as a novel mechanism to generate antibodies against P. falciparum RIFIN antigens on infected erythrocytes (Tan et al., Nature 2016). These templated insertions potentially add a novel biological mechanism used by the immune system to generate B-cell receptor repertoire diversity.

**Aims:** We investigated whether templated insertions occur in the B-cell repertoire of healthy donors and whether such insertions could be functionally expressed to explore their biological function.

**Methods:** We obtained >52,000 unique full-length VDJ sequences of IgM, IgG, IgA, and IgE isotypes by unbiased ARTISAN PCR (Koning et al., BJH 2016) from 6 healthy donors. Abnormally long sequences and junctions were searched for templated insertions by BLAST. Identified VDJ carrying templated insertions were co-expressed with a panel of 172 light chains on multiple myeloma cell lines and assessed for surface expression of transgenic immunoglobulin. The VDJ described by Tan et al. were included as controls.

**Results:** Six unique VDJ sequences, all from the same donor, carried a templated insertion in-frame (E=10-37–0). These sequences represented all VDJ sequences from 6 healthy donors. Abnormally long sequences and junctions were searched for templated insertions by BLAST. Identified VDJ carrying templated insertions were co-expressed with a panel of 172 light chains on multiple myeloma cell lines and assessed for surface expression of transgenic immunoglobulin. The VDJ described by Tan et al. were included as controls.

**Summary/Conclusions:** We have developed i) advanced microscopy techniques allowing multiscale 3D visualization of entire bone marrow cavities with cellular and subcellular detail ii) customized computational tools enabling the detection and quantification of discrete cell subsets/structures in 3D images of the BM in an unbiased fashion, as well as a rigorous spatial statistical analysis of cellular interactions.

**Results:** Using 3D-quantitative microscopy (3D-QM) we uncover that BM stromal cells are in fact 15-20 fold more abundant than previously reported. The massive underestimation of these relevant cell subsets results from the highly inefficient isolation of these cellular types with currently employed flow cytometry protocols. Our image-based analyses further reveals that sinusoidal and CAR cell stromal networks occupy a disproportionately large fraction of the BM space, consequently constraining the tissue volume available for hematopoietic cell distribution. In fact, the vast majority of BM resident hematopoietic cells are unavoidably in direct contact with the CAR cellular projections and in close proximity with the sinusoidal surface.

**Summary/Conclusions:** Collectively, our quantitative description of stromal microarchitecture, challenges current models of cell type-specific niche interactions in the BM, which are based in largely inaccurate estimations of cell frequency and spatial confinement of stromal cells in this organ.

**P268**

**TARGETING THE CASPASE / NOX2 AXIS TO MODULATE MACROPHAGE POLARIZATION**

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**Background:** Caspases, which are key effectors of apoptosis, have demonstrated non-apoptotic functions. One of these functions is the differentiation into macrophages of peripheral blood monocytes exposed to Colony-Stimulating Factor-1 (CSF1). Conversely, GM-CSF induces the differentiation of monocytes into macrophages in a caspase-independent manner. Macrophages generated by CSF1 and GM-CSF have distinct polarity.

**Aims:** Macrophage polarization plays an important role in the pathogenesis of various human diseases as cancer, leading us to explore if caspase inhibition would affect macrophage polarization.

**Methods:** To explore the role of caspases in CSF1 differentiation, we used human monocytes sorted from buffy coats or from blood of NOX2-deficient patients treated by cytokines, and we generated monocyte-restricted caspase-8 knockout and caspase-8 and caspase-10 double knockout mice, which were treated with bleomycin to induce pulmonary fibrosis.

**Results:** Caspase activation is involved in the generation of M2 polarized macrophages. Caspase inhibition delays the ex vivo differentiation of peripheral blood monocytes exposed to CSF1 and modifies the phenotype of generated macrophages, e.g. cell shape, surface markers and cytokine secretion. In mice, caspase knock-out also modified the phenotype of monocytes induced to differentiate into macrophages. Caspase activation appeared to be prominent at the mitochondria level and responsible for the NOX2-dependent generation of cytosolic radical oxygen species (ROS). Activation of the NOX2 complex is associated with p47phox cleavage by caspases. Mice treated with bleomycin typically develop a pulmonary fibrosis. Bleomycin-induced lung fibrosis was delayed in monocyte-restricted caspase-8 knockout mice and prevented by treatment with a caspase inhibitory molecule, including zVAD-fmk and the clinically developed IDN6556. This effect was associated with a change in the polarisation of lung-infiltrating macrophages.

**Summary/Conclusions:** Caspase inhibition in monocytes prevent the development of bleomycin-induced lung fibrosis by modifying macrophage polarization, suggesting that caspase inhibitory molecules may be an exciting thera- peutic strategy to modulate macrophage polarization with diverse applications including cancer treatment.
clastogenic assays were used to elucidate the down-stream effects of the elevated CXCL13. Recombinant CXCL13 protein as well as medium produced by co-cultured MM-Mφ increased RANKL expression and induced TRAP+ osteoclast (OC) formation in vitro, while CXCL13 neutralization blocked these activities. We next abrogated CXCL13 expression in MM cells using the CRISPR/Cas9 technology. The loss of CXCL13 had no effect on MM in vitro growth or drug sensitivity. However, mice inoculated with CXCL13-silenced MM cells developed significantly weaker BM disease compared to mice receiving the non-manipulated cells. Reduced tumor load correlated with decreased numbers of M2c-MΦ in BM, decreased bone disease, and lower expression of OC-associated genes. Finally, the presence of CXCL13 in primary MM samples was evaluated. B cells and levels of CXCL13 transcript and protein were detected in BM aspirates from MM patients (n=24) in comparison to normal BM (n=5) and were in correlation with gene expression signature associated with OC activation and M2c MΦ phenotype (Figure 1).

Summary/Conclusions: Our findings suggest that bidirectional interactions of MΦ with MM tumor cells result in M2c-MΦ polarization, CXCL13 induction and subsequent OC activation, enhancing their ability to support bone resorption and MM progression. CXCL13 may thus serve as potential novel target for the diagnosis and treatment of MM.

Figure 1.

P270

RE-ORDERING THE B CELL DEVELOPMENT HIERARCHY IN HUMAN FETAL BONE MARROW: CHARACTERISATION OF A NOVEL HUMAN FETAL B PROGENITOR

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Background: The cellular hierarchy of normal human fetal B-lymphopoiesis remains poorly defined. We have previously identified a novel population of PreProB progenitors (CD34+CD19+CD10-) in fetal liver (FL) [1] that is further expanded in fetal bone marrow (FBM) [2], and co-exists with adult-type CD34+CD19+CD10+ ProB progenitors. Increasing evidence indicates that infant ALL, and many cases of childhood ALL arise in fetal life, suggesting that ontogeny-related changes in B-cell development may be important for in utero leukaemia initiation. Therefore, understanding the human fetal B cell hierarchy, especially the differences between PreProB and ProB progenitors may be key to understanding the pathogenesis of infant and childhood leukaemias. Aim: To define B cell developmental markers early in second trimester FBM, with a view to establishing the fetal B cell hierarchy.

Methods: The characteristics of the haematopoietic stem cell (HSC), lympho-myeloid multipotent progenitor (LMPP), early lymphoid progenitor (ELP) and committed B-progenitor compartments of FBM samples were analysed by multiparameter flow cytometry. Gene expression analysis and clonogenic assays, transcriptome analysis and single cell RQ-PCR.

Results: All stages of B cell development were demonstrable in human FBM up to transitional B cells with a rapid expansion of B progenitor numbers from the LMPP stage. FBM HSC, progenitors (MPP, LMPP, ELP, PreProB and ProB-progenitors) and B-cells were FACS-sorted for functional/molecular assays. Functional assays: B cell differentiation assays demonstrated expected multi-lineage output from HSC/MPP, and LMPP, but a pure B cell output from PreProB and ProB progenitors. While PreProB progenitors gave rise to ProB progenitor cells in vitro, the converse was not true; thereby placing PreProB upstream of ProB in the B cell hierarchy. Myeloid colony assays gave expected multilineage output from HSC/MPP and GM colonies from LMPP, while ELP, PreProB and ProB progenitors generated no colonies, confirming their lymphoid commitment. Transcriptome analysis and single cell RQ-PCR: Fetal HSPC populations were flow sorted and analysed by RNAseq of bulk populations as well as single cell RQ-PCR using a customised 96-gene panel. Expression of HSC, MPP and LMPP showed good spatial segregation from lymphoid progenitors (ELP, PreProB and ProB) by principal component analysis both at bulk and single cell level. Single cell analysis demonstrated a differentiation trajectory from HSC to mature B cells with PreProB progenitors clustering between LMPP and ProB. These results were consistent with the model that specific genes from HSC to ProB progenitors and the level of expression was always lower in PreProB compared to ProB, suggesting they are upstream of ProB. PreProB progenitors demonstrated a distinct gene expression profile compared to ProB progenitors. 739 genes were significantly differentially expressed between two populations, with 503 of these being upregulated in PreProB, including some myeloid and leukaemia-associated genes. Genes overexpressed in ProB included B cell specific genes.

Summary/Conclusions: Detailed immunophenotypic, functional and molecular studies allow us to propose a human fetal B cell developmental hierarchy for the first time in which the unique PreProB progenitors are distinct from and lie upstream of the ProB progenitors. These results may have important implications in understanding the pathogenesis of infant and childhood leukaemias.


P271

HUNDREDS OF EMBRYONIC HEMATOPOIETIC PRECURSORS CONTRIBUTE TO LIFE-LONG HEMATOPOIESIS

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Background: Prior studies of the frequency of emerging hematopoietic stem cells (HSCs) and their precursors during mammalian ontogeny have all required ex vivo expansion, transplantation or co-culture of fetal tissues. Here, we employed the Cre/Confetti allele, in which a cassette targeted to +/+Vav1 (mid-gestation endothelial precursors, E7), ROSA26(Confetti+Flk1+/Cre) (mesodermal precursors, E7), ROSA26(Confetti+/VegfCre) (mesodermal endothelial precursors, E8.5-E10.5), and ROSA26(Confetti+/Vav1+/Cre) (hematopoietic progenitors E11.5-E14.5). This correlation was used to estimate the number of hematopoietic precursors emerging during each stage of development. Aim: To determine the frequency of emerging HSCs and their precursors throughout mammalian ontogeny.

Methods: Here, we employed the Confetti allele, in which a cassette targeted to the ROSA26 locus randomly and permanently marks cellular progeny with GFP, YFP, RFP or CFP when exposed to Cre recombinase. We determined empirically that sample-to-sample variance in the distribution of Confetti colors in the blood of the following adult mice inversely correlated with the number of hematopoietic precursors specified during the indicated window of Cre recombinase activity: ROSA26(Confetti+Fik1+/Cre) (mesodermal precursors, E7), ROSA26(Confetti+/Flk1+Cre) (hematopoietic precursors, E8.5-E10.5), and ROSA26(Confetti+/Vav1+Cre) (hematopoietic progenitors E11.5-E14.5). This correlation was used to estimate the number of hematopoietic precursors emerging during each stage of development.

Results: An inverse correlation of sample-to-sample variance in the distribution of Confetti colors and numbers of labeled initiating events was observed using the sample-to-sample Confetti color variance in the blood of cohorts of ROSA26(Confetti+)+/+ and ROSA26(Confetti+)Vav1+/- mice (E11.5-E14.5). This correlation was used to estimate the number of hematopoietic precursors emerging during each stage of development.

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Summary: Our data further suggest that a developmental bottleneck exists after the fetal liver stage of hematopoietic ontogeny that restricts the numbers of precursors that ultimately contribute to life-long hematopoiesis.
Background: Mature immune tolerance requires the production of Foxp3 expressing regulatory T cells (Treg) cells in the thymus. Activation of NF-κB transcription factors is critically required for Treg cell development, partly via initiating Foxp3 expression. NF-κB activation is controlled by a negative feedback regulation through the ubiquitin editing enzyme α20, which reduces pro-inflammatory signaling in myeloid cells and B cells. In naive CD4+ T cells, A20 prevents necroptosis and promotes inflammation.

Aims: This study is aimed at analyzing the role of the NF-κB regulator A20 in Treg cell development and function.

Methods: We used A20F/FCD4Cre mice, which specifically lack A20 in T cells, to analyze the Treg cell compartment in vivo. We characterized expansion and differentiation of A20-deficient Treg cells in vitro. We performed competitive bone marrow engraftment between WT and A20-deficient bone marrow in vivo to analyze whether one bone marrow compartment would outperform another or would favor development of certain T cell or other immune cell subsets. We performed allogeneic hematopoietic stem cell transplantation with WT BM+T cells vs WT vs A20-deficient Treg cells to analyze whether A20-deficient Treg cells would reduce GVHD to the same extent as WT Treg cells.

Results: Using mice deficient for A20 in T lineage cells, we show that thymic and peripheral Treg cell compartments are quantitatively enlarged due to a cell-intrinsic developmental advantage of A20-deficient Treg cells. A20 deficient Treg cells efficiently suppressed effector T cell mediated graft-versus-host disease after allogeneic hematopoietic stem cell transplantation, demonstrating normal suppressive functionality. Holding thymic production of natural Treg cells in check, A20 thus integrates reduced regulatory T cell activity and increased effector T cell survival into an efficient CD4+ T cell response.

Summary/Conclusions: In light of the largely anti-inflammatory effects that have been attributed to A20 in many cell types, this proinflammatory aspect of A20 physiology in effector and regulatory CD4+ T cells is particularly important since it may contribute to a change of perception of the functions of A20 as a negative regulator of NF-κB in the context of inflammation. Whether targeted modulation of A20 activity allows the induction of Treg cell mediated immune tolerance or, alternatively, boosting of favorable T cell immunity is a question of translational relevance that needs to be addressed in the future.

P273

THE TRANSCRIPTION FACTOR C/EBPΩ REGULATES MAST CELL DEVELOPMENT AND FUNCTION

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Background: Mast cells are key effector cells involved in protection against infection and allergic responses. Defects in mast cells are related to immunologically disorders, and therefore it is critical to fully understand the transcriptional network that controls their generation and activity. Differentiation of progenitors to mature mast cells is promoted by several transcription factors, such as GATA1, GATA2, STAT5, and MITF, and requires downregulation of C/EBPα. Recently, we identified another member of the C/EBP family of transcription factors, C/EBPΩ, as a direct C/EBPα target gene. However, the role of C/EBPΩ in mast cells remains so far elusive.

Aims: In this study we aim to determine the role of the transcription factor C/EBPΩ in mast cell development and function. Next, we investigate the mechanisms by which C/EBPΩ is controlling these functions.

Methods: In order to determine the role of C/EBPΩ in murine mast cells, we generated Cebpg conditional knockout mice, which allow excision of Cebpg in the hematopoietic system from the early embryogenesis. We employed Cebpgfl/fl Vav-1Cre+ and Cebpgfl/fl Vav-1Cre- mice, referred here as WT and Cebpg KO, respectively. Excision of Cebpg was assessed by RT-PCR and western blot analysis in bone marrow and spleen cells. Using flow cytometry, we enumerated mast cell counts in the peritoneal cavity of healthy WT and Cebpg KO mice. Analysis of peritoneal cavity of WT and Cebpg KO mice showed similar frequency and numbers of mast cells in steady state conditions. However, Cebpg deficient mice exhibited increased number of peritoneal mast cells after LPS stimulation in comparison to WT controls. Functionally, we demonstrated that deletion of Cebpg reduced mast cell migration towards antigen, SCF or PGE, and impaired degranulation upon FcγRI-mediated activation. Further, BM-MNCs exhibited increased expression of C/EBPΩ in the absence of C/EBPΩ.

Summary/Conclusions: In summary, we revealed C/EBPΩ as an important transcription factor which suppresses C/EBPα expression, thereby favoring mast cell development and function. Our data identifies a new component of the mast cell transcriptional network and provides a better understanding of mast cells in normal physiological conditions and disease.
Hodgkin lymphoma

P275

LONG-TERM OUTCOME OF PATIENTS WITH NODULAR LYMPHOCYTE-PREDA NINTED Hodgkin lymphoma TREATED WITHIN THE RANDOMIZED HD7-HD15 TRIALS: AN ANALYSIS FROM THE GERMAN HODGKIN STUDY GROUP

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Background: Nodal lymphocyte-predominant Hodgkin lymphoma (NLPHL) is a rare entity accounting for approximately 5% of all Hodgkin lymphoma (HL) cases. Pathological and clinical features differ from classical HL (cHL). Pathologically, the malignant lymphocyte predominant (LP) cells stain consistently positive for CD20 and are negative for CD30. Clinically, NLPHL often has a rather indolent course. Despite of these differences, the first-line treatment of NLPHL is mostly very similar to cHL. However, analyses on the long-term course of patients with NLPHL who were treated identically to cHL are scarce.

Aims: To shed more light on characteristics and outcome of NLPHL patients treated identically to cHL, we performed an analysis using the database of the German Hodgkin Study Group (GHSG).

Methods: A total of 471 patients with NLPHL who had received first-line treatment within the randomized GHSG HD7-HD15 trials for newly diagnosed HL were identified. The studies were conducted between 1993 and 2009. Patients at all stages (early favorable: HD7, HD10, HD12; early unfavorable: HD8, HD11, HD14; advanced: HD9, HD12, HD15) were included.

Results: Among the 471 NLPHL patients, the median age was 39 years; 76% of patients were male; 53% of patients had early favorable, 16% had early unfavorable and 31% had advanced-stage disease. Study treatment consisted of ABVD- or BEACOPP-based chemotherapy alone, radiotherapy (RT) alone or combined-modality treatment (CMT). After a median observation of 9.2 years, the 8-year progression-free survival (PFS) rate for the whole patient group was 81.3% (83.2% for early favorable, 85.2% for early unfavorable, 76.2% for advanced stages). 80 of 471 patients (17%) had relapsing disease or relapsed during the course of follow-up (primary disease progression: 24 patients; early relapse: 8 patients; late relapse: 66 patients). Second malignancies including histological transformation into aggressive B-cell non-Hodgkin lymphoma (NHL) occurred in 48/471 patients (10%) (solid tumor: 25 patients; leukemia: 7 patients; NHL: 13 patients; unspecified malignancy: 4 patients). For all 471 patients included in the present analysis, the 8-year overall survival (OS) rate was 91.5% for early favorable, 98.6% for early unfavorable, 87.4% for advanced stages). A total of 43 deaths were observed during follow-up resulting in a death rate of 9%. However, only a minority of these deaths was NLPHL-related (n=10). In contrast, most patients died from second malignancies (n=20) or due to other causes (n=13) such as heart failure and lung disease.

Summary/Conclusions: Taken together, the results from this large analysis on NLPHL patients prospectively treated and followed within randomized clinical studies for newly diagnosed HL indicate an excellent lymphoma-specific outcome. Nonetheless, further treatment optimization is necessary as the majority of these deaths were not directly related but due to second malignancies or other treatment-related late effects. Thus, future clinical trials including NLPHL patients should evaluate whether it is possible to reduce the treatment intensity without compromising efficacy. This goal may be achieved by the partial replacement of conventional chemotherapy by targeted drugs such as anti-CD20 antibodies as well as the reduction of RT fields and doses.

P276

ADVANCED HODGKIN LYMPHOMA IN THE EAST OF ENGLAND CANCER NETWORK: A 10-YEAR COMPARATIVE ANALYSIS OF OUTCOMES FOR ABVD AND ESCALATED-BEACOPP TREATED PATIENTS Aged 16–59

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Background: The majority of young patients with advanced-stage Hodgkin lymphoma (HL) in the UK are managed with ABVD. However, following publication of the HD10 trial results in 2009, escalated-BEACOPP (escB) was introduced by some UK centres to improve disease control in poor-risk patients.

Aims: We present a 10-year retrospective multicentre analysis for advanced-stage HL patients, aged 16–59, diagnosed between 2004–2014 in the East of England Cancer Network and treated predominantly outside of clinical trials. Our study period includes the 5 years before and after, the introduction of escB. We estimated the 5-year progression-free survival (PFS) and overall survival (OS) rates for the whole cohort, and treatment subgroups, to assess the impact of escB on survival outcomes.

Methods: We collected data retrospectively from 8 hospitals in the East of England Cancer Network from a referral population of 2.64 million (incidence: 0.95 cases per 100,000). Six of the 8 centres introduced escB for poor-risk patients, as determined by physician and patient choice; 44 patients were treated with escB, 202 with ABVD, 3 with alternative regimens, and 1 died pre-treatment. The median age at diagnosis was 35 years (range: 16–59) and the median follow-up was 4 years (range: 0.3–9.7). The 5-year PFS for all patients was 82% and 5-year OS was 92%. There was evidence of a physician–patient preference to treat poor-risk patients with escB, as a greater proportion of escB patients had a high international prognostic score (IPS 3+) than in ABVD patients (escB 75% vs ABVD 38%, p<0.0001). For the whole cohort, PFS was better for patients treated with escB compared with ABVD (5-year PFS escB 95% vs ABVD 80%; HR 4.3 (95% CI:1.97–9.7), p=0.0261), but there was no difference in OS (5-year OS escB 97% vs ABVD 92%; HR 2.6 (95% CI:0.69–10.4), p=0.312). However, patients with IPS 3+ had both a PFS and OS advantage when treated with escB compared with ABVD (5-year PFS escB 95% vs ABVD 80%; HR 9.24 (95% CI:1.43–24.89), p=0.012; 5-year OS escB 94% vs ABVD 84%; p=0.0325). Twenty-nine ABVD patients and 3 escB patients had at least 1 subsequent stem cell transplant (including 6 allografts post-ABVD and 3 allografts post-escB), and there was equal use of consolidation radiotherapy between regimens (11% of both ABVD and escB patients). Treatment-related toxicities were less common in the escB arm. Only 1 of the 20 (6.7%) pre-menopausal women treated with escB died <30 years at diagnosis regained menstrual periods during follow-up, 5 (45.5%) of whom subsequently conceived (including 6 live births, 1 miscarriage, and 1 termination). One of the 6 (16.7%) pre-menopausal women aged 30–39 years did not regain menstrual periods, which were not sustained beyond 3 years’ follow-up.

Summary/Conclusions: Our data reflect clinical trials results which indicate a first-remission PFS but not OS advantage for unselected young advanced-stage HL patients treated with escB compared with ABVD. However, our data strongly suggest that patients with a poor IPS score derive a PFS and OS benefit from treatment with escB compared with ABVD.
months after treatment discontinuation in both groups. Relapses were documented by histologic examination in both groups. When relapse was documented all patients received salvage therapy with high dose chemotherapy (DHAP), for at least two courses, followed, in case of CR, by ASCT.

**Results:** After a median 62-months observation (range, 4–108), 83 patients, evenly distributed in the two groups, had a relapse of disease. Of these, 28 of 43 patients (67.4%) of the historical cohort vs 17 of 40 patients (42.5%) of the imaging cohort, showed a larger spread of disease at restaging, i.e. stage superior to IIb, and a more frequent extranodal involvement, 10/43 (23.3%) patients in the historical group vs 3/40 (7.5%) patients in the imaging group (p=0.01). Furthermore, if we considered only asymptomatic patients, one recurrence was detected in 26 of 43 patients in the imaging group and 17 of 40 patients in the historical group, p=0.02. CR rate with second line therapy were higher in the imaging group (27, 67.5%) compared with the historical group (19, 44.2%; p=0.032). The 3-years DFS was 75% in the imaging group and 36% in the historical group, p=0.02.

**Summary/Conclusions:** This is the first prospective case-control study using SMAP-US plus CXR to monitor patients with advanced stage HL. We show that SMAP-US plus CXR is a valuable tool to improve follow-up in patients with a high risk of recurrence. Our data indicate that the early detection of HL recurrence allows to begin rescue therapy in patients with a more limited disease and, consequently, increase its effectiveness in terms of probability to response and DFS.

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**P278**

**LATER LINE DRUG TREATMENT PATTERNS OF CLASSICAL HODGKIN’S LYMPHOMA PATIENTS IN CANADA, FRANCE, GERMANY AND THE UNITED KINGDOM**

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**Background:** Whilst chHL is seeing increasing ‘cure’ rates, a cohort of patients remain who, due to multiple relapses, require 3rd or 4th line treatments. Real world treatment patterns for RRHL patients are currently less understood.

**Aims:** To understand the drug treatment patterns of chHL patients in 3rd or later lines.

**Methods:** Real-world data were collected through a cross-sectional survey administered to physicians in Canada (Ca), France (Fr), Germany (Ge), and the UK between May and Sep 2016. Physicians provided data on the last 8 chHL patients receiving 3rd or 4th line drug treatment. Data captured included demographics, disease history and treatment patterns. Auto/allo stem cell transplants (auto/alloSCT) were not classified as a treatment line and limited data was available to determine when a SCT was received. Summary statistics were reported and differences between sub-groups assessed using chi-square tests.

**Results:** In total 116 physicians (Ca, 16; Fr, 31; Ge, 44; and UK, 25) provided information on 959 chHL patients (Ca, 128; Fr, 243; Ge, 351; and UK, 237) on 3rd or later lines of drug treatment. Data for 954 chHL patients on 3rd line drug treatment was captured. Patients had a mean age of 54.0 years (SD: 16.79) at the point of data capture. 57% were male; 43% female. 30% had bulky disease. 84% of patients had been tested for the Epstein Barr virus (EBV), 36% confirmed positive. The most commonly prescribed 3rd line drug treatment was a brentuximab-vedotin (BV) based regimen (35%). BV use was significantly different across the markets; Canada (34%), France (35%), Germany (30%) and the UK (44%) (p=0.010). The next most commonly prescribed 3rd line treatment was BEAM (7%) and bendamustine (7%). 4% of 3rd line patients received a PD-1 inhibitor. Of 3rd line BV patients the majority received ABVD (69%) or BEACOPP (19%) at 1st line. Their most common 2nd line drug treatments were DHAP (21%), ICE (10%), ESHAP (9%) and BEACOPP (9%). 59% of all 3rd line BV patients had undergone an auto/alloSCT at some point during their treatment history. Of 3rd line patients receiving non-BV-based regimens 6% had been treated with BV previously (1st/2nd line). Of 3rd line patients treated with a PD-1 inhibitor 7% had been previously treated with BV. Data for 453 chHL patients on 4th line drug treatment was captured. 4th line patients had a mean age of 55.5 years (SD: 16.79) at the point of data capture. 56% were male; 44% female. 83% had been tested for EBV, 38% confirmed positive. 30% of 4th line patients received a BV based regimen – BV use across markets was significantly different; Canada (20%), France (38%), Germany (23%) and the UK (36%) (p=0.007). At 3rd line this cohort had mostly received DHAP (16%), BEAM (15%) or ICE (11%). 5% of 4th line BV patients also received a BV based regimen at 3rd line. 12% of 4th line patients received a BV regimen at 3rd line. At 4th line 38% of this cohort received a PD-1 inhibitor, 19% bendamustine and 9% gemcitabine.

**Summary/Conclusions:** Real-world data indicates an unmet medical need for chHL patients with multiple relapses, reinforced by the use of PD-1 inhibitors in those relapsing post BV based regimen at 3rd line. There also appears to be no clear standard of care at 3rd line, again highlighted by use of a range of regimens and PD-1’s. This study was sponsored by Bristol-Myers Squibb.

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**P279**

**CHEMOTHERAPY AND RADIATION IMPROVE SURVIVAL IN EARLY STAGE CLASSICAL HODGKIN LYMPHOMA, A STATEWIDE CANCER REGISTRY ANALYSIS**

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**Background:** Early stage classical Hodgkin Lymphoma (cHL) has been shown to have an excellent outcome. Recent studies have therefore focused on decreasing the toxicity that results from the addition of radiation therapy to chemotherapy. However, it remains unclear whether omitting radiation as part of the initial therapy of cHL is associated with a similar survival outcome.

**Aims:** The primary aim of this study is thus to investigate the outcomes of chHL patients treated in a statewide cancer registry for chHL patients treated with chemotherapy alone vs patients treated with both chemotherapy and radiation.

**Methods:** All adult patients (older than 18) diagnosed with cHL in Kentucky Cancer Registry (KCR) from 2005-2014 were retrospectively reviewed. Baseline characteristics including age at diagnosis, gender, histology, stage, B symptoms, extranodal involvement, and the site involved were collected. First line treatment modalities as well as overall survival outcomes were reviewed. Stage I and II patients without B symptoms were considered favorable, whereas those with B symptoms were considered unfavorable. Patients with stage III and IV disease were given an advanced stage designation. To adjust for selection bias, patient deaths during the first 6 months of diagnosis were censored for overall survival analysis.

**Results:** A total of 961 patients were identified. Median age was 41 (range 18-91) and 60.9% (n=585) were younger than 50. The group included a mild predominance of males (55.5%). Only 1.7% (n=16) had extranodal involvement at presentation. Of those with known histology (78.8%), the most common was nodular sclerosis (71.2%), followed by mixed cellularity (22.8%), lymphocyte rich (3.8%) and lymphocyte depleted (1.9%). Median follow up time was 45 months (range 0-136). The 10-year overall survival for the favorable group (n=329) was 77% (95% CI: 71.1-88.8) versus 68% for the unfavorable group (n=144) and 42% for the advanced group (372) (p<0.001).

There was no statistical difference in survival between stage I (n=170), and stage II (n=385) disease (p=0.99). Treatment modalities were then compared for the favorable risk group alone. Those who received chemotherapy alone (n=145) were compared to those who received combined chemotherapy and radiation (n=148) as their primary therapy. The 10-year overall survival for the cohort receiving chemotherapy and radiation was 87% compared to 75% for those receiving only chemotherapy (p<0.001) (Figure 1). When adjusted by multivariate analysis for risk factors affecting 10 year survival of the favorable cohort, only age <50 and the treatment modality were independently associated with a statistically significant difference in overall survival (HR of 0.11 (p=0.001) and 3.94 (p=0.001), respectively).

**Figure 1.**

**Summary/Conclusions:** Our large data cohort shows the presence of B symptoms observed in a statewide registry for cHL patients treated with chemotherapy alone vs patients treated with both chemotherapy and radiation. There was a worse prognosis than the number of nodal regions involved for early stage disease. Although the use of radiation as part of initial therapy for early stage disease might have increase long term toxicity, it continued to provide superior survival at 10 years.
THE IMPACT OF TREATMENT WITH BRENTUXIMAB VEDOTIN ON OVERALL SURVIVAL OF PATIENTS WITH HODGKIN LYMPHOMA RELAPSED AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION. A NATIONWIDE POPULATION BASED ANALYSIS


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Background: Patients with Hodgkin Lymphoma (HL) who relapse after autologous Stem Cell Transplantation (auto-SCT) have a dismal prognosis. Advanced disease stage, presence of B-symptoms, extranodal involvement at the time of relapse and duration of remission of less than 12 months are parameters associated with decreased overall survival (OS). Brentuximab Vedotin (BV), an anti-CD30 monoclonal antibody conjugated to a microtubule-disrupting agent, has shown clinical efficacy in HL. Although in the setting of post-auto-SCT relapse, BV produces an overall response rate of approximately 75% with a median progression free survival (PFS) of 9 months, the impact of BV on OS has not been addressed in previously published studies.

Aims: To examine the impact of treatment with BV on OS of patients with HL relapsed after auto-SCT.

Methods: Data for patients with HL who underwent auto-SCT in Greece during the last 20 years were collected. Study group consisted of 214 patients who experienced post-auto-SCT relapse. In order to examine the impact of BV on OS, patients were divided in 2 cohorts depending of the date of BV availability in Greece (January/2013). Cohort 1 consisted of 178 patients who relapsed before January/2013, while Cohort 2 consisted of 36 patients relapsed after BV became available. Patient’s characteristics are shown in Table 1.

Table 1. Patients characteristics.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cohort 1</th>
<th>Cohort 2</th>
<th>Significance</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>35 (14-84)</td>
<td>40 (16-85)</td>
<td>ns</td>
</tr>
<tr>
<td>Sex</td>
<td>120 (67)</td>
<td>12 (33)</td>
<td>ns</td>
</tr>
<tr>
<td>B-symptoms (yes vs no)</td>
<td>10 (6)</td>
<td>11 (6)</td>
<td>ns</td>
</tr>
<tr>
<td>Stage (III vs IV)</td>
<td>7 (4)</td>
<td>0 (0)</td>
<td>ns</td>
</tr>
<tr>
<td>Extramalinal involvement (yes vs no)</td>
<td>13 (8)</td>
<td>10 (6)</td>
<td>ns</td>
</tr>
<tr>
<td>Time from last to relapse (≤12 months)</td>
<td>10 (6)</td>
<td>10 (6)</td>
<td>ns</td>
</tr>
<tr>
<td>Response (CR vs PR)</td>
<td>16 (9)</td>
<td>10 (6)</td>
<td>ns</td>
</tr>
</tbody>
</table>

The following variables were included in a multivariate Cox proportional hazard regression analysis model: 1) age of patient, 2) Sex, 3) B-symptoms (yes vs no), 4) Stage of disease (I-II vs III-IV), 5) extranodal disease, 6) time from auto-SCT to relapse (≤12 vs >12 months), 7) Relapse before or after BV availability (Cohort 1 vs Cohort 2). In order to exclude any confounding effect of subsequent treatments, analysis was performed by censoring patients at the time of alloengenic SCT or treatment with immune checkpoint inhibitors (IC-inhibitors).

Results: In multivariate analysis the following variables were statistically associated with OS: 1) The presence of B-symptoms [HR=2.07, (95% CI, 1.39-3.07), p<0.001] and 2) Relapse in less than 12 months after auto-SCT [HR=3.07, (95% CI, 1.39-6.92), p=0.001)], were associated with decreased OS, while 3) Response after first salvage [HR=0.46, (95% CI, 0.31-0.68), p<0.001], and 4) BV availability [HR=0.36, (95% CI, 0.16-0.79), p=0.011] were associated with increased OS (Figure 1). Similar results were obtained when analysis was performed without censoring patients at the time of allo-SCT or treatment with IC-inhibitors (data not shown). Summary/Conclusions: Patients in Cohort 2 survived longer even when censored for allo-SCT or treatment with IC-inhibitors. All patients in Cohort 2 treated with BV while only 18% of patients in Cohort 1 received treatment with BV. The results of our study strongly suggest that BV improves OS in patients with HL relapsed after auto-SCT. To our knowledge this is the first study showing an OS advantage of treatment with BV.
tion was defined as imaging at or before week 12 of treatment, whereas late radiological evaluation was performed at or after week 16. Response evaluation was performed according to the Lugano Classification and its update regarding immunomodulatory therapy.

Results: Between 06/2015-11/2016, 87 patients were enrolled in a name-based program in Turkey. Two, 19, and 3 patients who had not yet received nivolumab, had not reach the time for early radiological evaluation, and who died before any radiological evaluation were excluded from the analysis. Thus, 63 patients from 23 centers were retrospectively analyzed. Median follow-up was 6 months, median age was 29 (18-75) and patients had a median 5 (2-11) previous lines of therapy. 44 patients (70%) had been treated by stem cell transplantation (SCT) and 45 (76%) patients had been treated by BV. The ORR was 68% with 15 CR (95%CI 0.020-0.28; CR 26%, PR 42%, SD 12%, PD 20%) among 59 patients evaluated in 12 weeks of nivolumab treatment. The ORR was 67% with 9 (24%) patients with CR after 16 weeks of treatment (95%CI 0.004-0.26; CR 24%, PR 43%, SD 6%, PD 27%). Estimated OS was 95% (95%CI 0.80-0.98) and estimated PFS was 71% (95%CI 0.65-0.82) at 12-months. Median OS was not reached, while, according to the late response rates, the median PFS was 14 months. However, it was only 3 months in patients with PD at the late radiological evaluation. Regarding responses to last treatment prior to nivolumab, we detected that 28 (67%) of 42 PD cases had objective early responses and 70% of PD cases had ORR in the late response evaluation (CR in 4, PR in 12 pts). 8 patients underwent transplantation following nivolumab. Among 5 patients who had been treated by allo-SCT, 4 had CR at the time of transplantation and they are alive with ongoing response. Safety profile was acceptable and only two patients required cessation of nivolumab due to serious adverse events: one due to autoimmune encephalitis and one due to aggravation of graft versus host disease. At the time of analysis, 40 cases were still on nivolumab treatment (64%). Among the 40 cases with early objective responses to nivolumab, 35 (88%) showed ongoing objective responses. All 24 cases with objective responses in the late evaluation had ongoing responses at the time of analysis (Figure 1).

Summary/Conclusions: In conclusion PD-1 blockers are new options to meet the unmet need in patients with cHL refractory to BV treatment, and possibly a bridge for these patients before transplantation.

P282

GENOTYPING OF HODGKIN LYMPHOMA ON THE LIQUID BIOPSY

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Background: In classical Hodgkin lymphoma (cHL) the low representation (1-5%) of Reed-Sternberg cells (RS) challenged tumor genotyping on the diagnostic tissue biopsy. Consistently, the mutational profile of newly diagnosed cHL is poorly characterized, and the genetics of refractory disease is completely unknown. Cell free DNA (cfDNA) is shed into the blood by tumor cells undergoing apoptosis and can be used as source of tumor DNA for the identification of somatic mutations. In addition cfDNA is representative of the entire tumor heterogeneity, thus allowing the identification of mutations from tumor cells residing in non-biopsied sites.

Aims: This study aims: i) at providing the evidence that the mutational profile of cHL can be tracked by using plasma cfDNA; and ii) at characterizing the genetics of newly diagnosed cHL and, for comparative purposes, of refractory cHL.

Methods: The study incudes 28 newly diagnosed cHL and 9 chemorefractory cHL. All cases were provided with cfDNA from plasma collected at baseline, before treatment start, and paired DNA from granulocytes as source of germline DNA to filter out polymorphisms and sequencing noise. Paired genomic DNA from formalin fixed paraffin embedded (FFPE) tumor tissue biopsies was available for 17 patients, including 3 cases for which RS enriched areas were macromodissected. A targeted resequencing panel optimized to include the coding exons and splice sites of 77 genes (192Kb) that are recurrently mutated in B-cell lymphomas was used for genotyping. Libraries were prepared from plasma cfDNA, germline gDNA and tumor gDNA according to the CAPP-seq targeted enrichment strategy (Nimblegen) and subjected to ultra-deep-next generation sequencing (NGS) on the MiSeq platform (Illumina). The sequencing was tailored to obtain a depth of coverage >2000x in ~80% of the target region in all samples, which allowed a sensitivity of 3x10^-3. The somatic function of VarScan2 was used to call non-synonymous somatic mutations, and a stringent bioinformatic pipeline was applied to suppress the background noise and to filter out sequencing errors.

Results: In newly diagnosed cHL, genotyping of plasma cfDNA identified non-synonymous somatic mutations in STAT6 (43%), TNFAIP3 (43%), ITPKB (32%) B2M (21%), GNA13 (14%), CIITA (7%), XPO1 (7%) and CD58 (4%) among the most recurrently affected genes (Figure 1A-B). In refractory cHL patients, genotyping of plasma cfDNA identified non-synonymous somatic mutations in ITPKB (44%), TNFAIP3 (33%), KMT2D (33%), B2M (33%), GNA13 (33%), XPO1 (22%), TET2 (22%), IKBKB (22%), BIRC3 (22%) and STAT6 (22%) among the most recurrently affected genes. Mutations of KMT2D (33%) and TET2 (22%) were enriched in refractory chL patients compared to newly diagnosed cases, suggesting that they contributed to the chemorefractory phenotype (Figure 1C-D). Using by highly sensitivity techniques, most of the mutations discovered in cfDNA were also identified in pair tumor DNA from the tissue biopsy and/or macromodissected RS cells, thus confirming their tumor origin (Figure 1F). By pathway analysis, the mutational profile pointed to the involvement of PI3K/AKT signaling, NF-kB signaling, TNFα signaling and the immune escape in cHL. ITPKB (a negative regulator of the PI3K/AKT signaling pathway) was specifically mutared in cHL across aggressive B cell lymphomas.
P283
FDG PET-CT MAYBE A USEFUL TOOL TO IDENTIFY DOXORUBICIN INDUCED CARDIOTOXICITY IN HODGKIN LYMPHOMA
G. Sambuceti1, E. Arboscio1, P. Spatarossa1, M. Miglino1, M. Bauckneht1, F. Fiz1, S. Morbelli1, G. Ferrarazzo1, A. Bellodi1, A. Da Col1, D. Avenoso1, F. Ballerini1, M. Bergamaschi1, L. Mitscheunig 1, M. Sarocchi1, C. Brunelli1, M. Gobbi1, R. Lemoli1
1IRCCS San Martino - IST, Genova, Italy

Performed this procedure underwent a 12 months cardiological follow-up assessment encompassing was manually drawn on the left ventricular myocardium. Average standardized ability of 4 consecutive PET/CT scan for staging (PET1), interim (PET2), post- treated following ABVD scheme were analyzed. Inclusion criteria were: 1) avail- diotoxicity.

PET/CT imaging might represent a useful tool to identify high-risk patients and damage can be preceded by an enhanced glucose uptake. 18F-FDG Summary/Conclusions: however, late follow-up ECHO detected the appearance of first-degree diastolic however, late follow-up ECHO detected the appearance of first-degree diastolic impairment with respect to baseline in 9 of the 25 examined patients (36%, 4 patients showed signs or symptoms potentially related to DXR cardiotoxicity. 18F-FDG PET/CT of 25 patients affected by Hodgkin Disease (HD), treated without any progressive increase. Accordingly, the ratio between PET4 and PET1 LV-SUV in the two subgroups was 3.85±0.8 and 1.06±0.4, respectively (p<0.001). Up to six months after therapy discontinuation, none of the 25 patients showed signs or symptoms potentially related to DXR cardiotoxicity. However, late follow-up ECHO detected the appearance of first-degree diastolic impairment with respect to baseline in 9 of the 25 examined patients (36%, 4 females, mean age 36±18). This finding occurred in 5/6 “increasers” (83%) and in only 4/19 non-increasing (21%) (p<0.001).

Summary/Conclusions: The present data indicate that DXR related myocardial damage can be preceded by an enhanced glucose uptake. 18F-FDG PET/CT imaging might represent a useful tool to identify high-risk patients and to implement personalized program to monitor and prevent DXR-induced card- diotoxicity.

P283
FDG PET-CT MAYBE A USEFUL TOOL TO IDENTIFY DOXORUBICIN INDUCED CARDIOTOXICITY IN HODGKIN LYMPHOMA
G. Sambuceti1, E. Arboscio1, P. Spatarossa1, M. Miglino1, M. Bauckneht1, F. Fiz1, S. Morbelli1, G. Ferrarazzo1, A. Bellodi1, A. Da Col1, D. Avenoso1, F. Ballerini1, M. Bergamaschi1, L. Mitscheunig 1, M. Sarocchi1, C. Brunelli1, M. Gobbi1, R. Lemoli1
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Background: Doxorubicin (DXR) induced cardiotoxicity is related to several mechanisms, including interference of mitochondrial respiratory chain and acceleration of glycolysis. We previously reported that this treatment may enhance myocardial FDG uptake. Aims: The present study aimed to verify whether this metabolic response on serial PET/CT imaging can predict myocardial function, non-invasively evalu- ated by follow-up echocardiography (ECHO). Methods: 18F-FDG PET/CT of 25 patients affected by Hodgkin Disease (HD), treated without any progressive increase. Accordingly, the ratio between PET4 and PET1 LV-SUV in the two subgroups was 3.85±0.8 and 1.06±0.4, respectively (p<0.001). Up to six months after therapy discontinuation, none of the 25 patients showed signs or symptoms potentially related to DXR cardiotoxicity. However, late follow-up ECHO detected the appearance of first-degree diastolic impairment with respect to baseline in 9 of the 25 examined patients (36%, 4 females, mean age 36±18). This finding occurred in 5/6 “increasers” (83%) and in only 4/19 non-increasing (21%) (p<0.001).

Summary/Conclusions: The present data indicate that DXR related myocardial damage can be preceded by an enhanced glucose uptake. 18F-FDG PET/CT imaging might represent a useful tool to identify high-risk patients and to implement personalized program to monitor and prevent DXR-induced card- diotoxicity.
Methods: A retrospective pre-post cohort study was conducted in pts switching from DFX DT to FCT using pharmacy and medical claims (06/2014 - 05/2016) from the Symphony Health Solutions' Integrated Dataverse (IDV®) database. Eligible pts were ≥2 years old, had a diagnosis of an inherited or acquired hematological disorder requiring transfusions (e.g., sickle cell disease, myelodysplastic syndrome), ≥2 DXF FCT claims (1st claim=index date), ≥2 DXF DT claims, ≥56 months of continuous clinical activity (between periods) pre-index. Medication possession ratio (MPR) (percentage of time with access to medication) was calculated for DXF DT during the "DFX DT period" (from earliest DXF DT claim to index date) and for DXF FCT during the "DFX FCT period" (from index date to end of data availability/ICT switch). Proportion of days covered (PDC) and persistence (without a gap ≥30 or 60 days between claims) were assessed in the DXF DT and DXF FCT periods over fixed intervals of 3 and 6 months, which started from the index date in the DXF FCT period, or dispensing date of the most recent DXF DT claim prior to the beginning of a 3- or 6- month interval in the DXF DT period. Comparisons between the two periods were made using the Wilcoxon sign-rank test for continuous data and McNemar’s test for dichotomized data.

Results: Of the 606 eligible pts, 56% were female, 64% were <35 years old, and 42% had transfusions during the baseline period. The median durations of the DXF DT and DXF FCT periods were 350.5 days and 290.2 days, respectively. Compared with adherence to DXF DT, adherence to DXF FCT was significantly improved across all measures. Mean MPR of DXF FCT vs DXF DT was 0.80 vs 0.76 (p<0.001); 60.9% pts had a mean MPR ≥0.8 during the DXF FCT period compared to 54.3% during the DXF DT period (p<0.01). Mean 3-month PDC of DXF FCT vs DXF DT was 0.83 vs 0.71 (p<0.001); 50.0% pts had mean 3-month PDC ≥0.8 during the DXF FCT period compared to 34.5% during the DXF DT period (p<0.001). The proportion of pts with 3-month persistence to DXF FCT vs DXF DT (without a gap ≥30 days) was 87.2% vs 63.4% (p<0.01). Similarly consistent and significant results for PDC and persistence were observed using a 6-month time interval and/or a 60-day gap between claims.

Summary/Conclusions: Adherence and persistence to ICT was significantly improved in pts who switched from DXF DT to DXF FCT. Reasons for switching, which may contribute to improved adherence, were not examined in this study. Nevertheless, since the majority of pts were already adherent to DXF DT, the improved adherence to ICT can be further augmented with this formulation. This real-world study complements the ECLIPSE trial results and supports previous evidence of improved adherence to DXF FCT.

P287

ASSESSMENT OF THE PERFORMANCE OF A WIDELY AVAILABLE T2*/R2* LIVER IRON CONCENTRATION METHOD USED IN CLINICAL PRACTICE IN A POPULATION OF THALASSEMIA PATIENTS


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Background: Measurements of liver iron concentration (LIC) by magnetic resonance imaging (MRI) have become established and validated in several research intensive centers. While the validity of spin density projection assisted (SDPA) R2-MRI together with a core laboratory service has been validated in routine clinical practice settings, methods relying on in-house establishments of data acquisition protocols and data analysis have not yet been validated in this way.

Aims: To determine the limits of agreement between measurements of LIC by a widely available T2*/R2* MRI method and a reference standard SDPA R2-MRI method in a routine clinical practice setting.

Methods: Thalassemia patients (n=60) referred by the National Institute of Hematology and Blood Transfusion, Hanoi, Vietnam for routine LIC measurement by MRI were prospectively recruited with informed consent. Patients were randomised to be scanned in either a Philips Ingenia or a Siemens Avanto 1.5T scanner. The LIC of each patient was measured twice, once by a T2*/R2* technique using an available software and protocols (Iron Health Calculator: http://www.ironcalculator.com) and once by SDPA R2-MRI using a quality controlled core laboratory data analysis service (FerrScan®). Analysis by the T2*/R2* data analysis method were blinded from the SDPA R2-MRI results and vice versa. Reported data were analysed using the statistical methods of Bland and Altman.

Results: A plot of the T2*/R2* LIC against the SDPA R2-MRI LIC (Figure 1) shows the vast majority of the data falling below the line of equivalence indicating that the T2*/R2* method is understimating the LIC relative to the SDPA R2-MRI validated reference standard. The geometric mean ratio of T2*/R2* LIC to SDPA R2-MRI LIC was 0.44 (95% CI 0.36–0.55) indicating severe underestimation of LIC by the T2*/R2* method. The geometric mean ratios of the two LIC measurements were significantly different for the two scanners (0.28 for Philips and 0.68 for Siemens, p <0.0001) indicating that the bias of the T2*/R2* method against the reference standard is not universal but is dependent on both/either scanner type and/or data acquisition method. Bland Altman analysis indicates that 95% of pairs of measurements are predicted to have ratios between 3.73 and 0.05 indicating a very large random variability between the T2*/R2* method and the reference standard. The performance of the T2*/R2* method predicting SDPA R2-MRI LIC values above the clinically relevant thresholds of 7 and 15 mg Fe/g dw is characterized in the Table 1 showing positive predictive values (PPVs) and negative predictive values (NPVs) together with their 95% CIs.

Table 1.
The data indicate that the T2*/R2* method of measurement of LIC is not safe for routine clinical measurement of LIC because of the extremely poor NPVs which could result in inappropriate clinical decision making. The severe discrepancies of the T2*/R2* method from the reference standard are likely caused by several factors including non-optimal curve fitting algorithms, lack of a method to identify non-analyzable data, and the use of a calibration curve from the literature generated from data acquisition and analysis methods different from those used locally. These or similar pitfalls are likely to be encountered in many MR centres using non-regulated MR methods of LIC measurement.

**P288**

**SIMILAR TRENDS IN RENAL FUNCTION AS MEASURED BY SERUM CREATININE DURING LONG-TERM IRON CHELATION TREATMENT WITH OR WITHOUT DEFERASIROX IN PATIENTS WITH TRANSFUSIONAL HEMOSIDEROSIS**

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**Background:** Regular transfusion and iron chelation therapy (ICT) are often indicated for patients with β thalassemia, sickle cell disease (SCD) and other anemias, and can be lifelong requirements. As most patients now survive into adulthood and many experience prolonged exposure to ICT, there is increased risk of age-, disease- or drug-related complications, including changes in renal function. Evidence suggests that some patients receiving ICT experience changes in markers of renal function, mostly within normal limits, non-progressive and reversible with dose reduction and/or interruption. Recently, we reported a retrospective analysis of patients with transfusion-dependent anemia during a decade of deferasirox treatment indicating stable and a lack of any progressive worsening of renal function (Origa R et al. Blood 2016).

**Aims:** To assess serum creatinine (Scr) during long-term deferasirox treatment in subgroups of Italian patients with transfusional hemosiderosis who participated in the deferasirox registration studies and were then followed retrospectively.

**Methods:** Italian patients with β thalassemia, SCD, myelodysplastic syndromes or other anemias who received ≥1 deferasirox dose in the registration studies (studies 105, 106, 107, 108 or 109), had ≥1 post-baseline (BL) Scr measurement, and had medical records available were included. Scr values were collected retrospectively in 3-month periods from registration trial end until the latest patient assessment. Primary endpoint was Scr over time. Scr values during the retrospective period were evaluated by subgroups: here we report those who received only deferasirox and those who received no deferasirox but ICT during the retrospective period.

**Results:** 282 patients were included in the retrospective study who received ≥1 deferasirox dose in registration studies; of these, during the retrospective period, 98 (35%) received only deferasirox (group A) and 82 (22%) received no deferasirox but other ICT (group B). In group A, mean (SD) age at first quarter was 25.9 (12.1) years and 36 (37%) were male; in group B, mean (SD) age at first quarter was 27.0 (10.9) years and 25 (40%) were male. The proportion of pediatric patients was 28% (n=27) in group A and 19% (n=12) in group B.

Mean (SD) duration of deferasirox exposure in group A was 7.5 (1.7) years; mean daily deferasirox dose was 202 (108) mg.

In both subgroups analyzed, mean Scr was within normal limits and remained stable over time during the retrospective period (Figure 1). Analysis in adults showed mean Scr values were stable over time. As expected in growing children who are gaining height and weight, pediatric mean Scr absolute values increased from baseline in proportion with an almost linear increase in muscle mass over time.
Methods: A cohort of 25 well characterized patients was analyzed. Eighteen were initially referred to our center for unexplained hyperferritinaemia (HF), two for proven iron overload (IO) by MRI, 2 for chronic hemolysis and 3 for aregenerative anemia. A set of phenotypic tests was systematically assessed, including CBC, reticulocyte count, serum haptoglobin and measure of the Liver Iron Content (LIC) by MRI. For all patients with HF, causes linked to hepatic disease, inflammation and cancers were ruled out and no other cause of iron overload was identified. Phenotypic investigations failed to clearly identify the cause of the disorder. Therefore, each patient was tested for a panel of 32 genes involved either in iron homeostasis or hereditary anemias, using NGS. Libraries were obtained using the Custom SureSelectXT Target Enrichment system (Agilent, Santa Clara Ca USA) and sequenced on a MiSeq platform (Illumina, San Diego, Ca, USA). Each deleterious variation was independently checked using conventional Sanger sequencing. Written informed consent was obtained from all the patients for NGS genetic analyses.

Results: Initial phenotypic reassessment allowed classifying the patients into 5 different groups: 1/ isolated hyperferritinaemia (n=11); 2/ HF and IO (MRI >90 µmole/g dry weight) (n=17); 3/ hereditary anemia (HA) without IO (n=2); 4/ HA and IO (n=2); 5/ aregenerative anemia with IO (n=3). Among patients with an initial diagnosis of iron disorder, the reticulocyte count identified 2 undiagnosed chronically fully compensated hemolysis. Systematic screening using the gene panel identified a total of 14 sequence variations of clinical significance in 9 different genes and 9 patients. An isolated mutation was found in 7 and 2 patients with an initial diagnosis of iron or of red cell disorder respectively. A combined anomaly of red cell and iron genes was identified in 3 patients who displayed IO and compensated hemolysis or anemia. Digemin involving an HFE C282Y/wt or C282Y/H63D genotype and another "iron gene" was also shown in 3 patients with IO without anemia or hemolysis. No sequence variation of clinical significance was found in the sequenced genes of eleven of the studied patients.

Summary/Conclusions: On the phenotypic point of view, the present study highlights the importance to check for hematological data (CBC and reticulocytes) in patients with HF, because this can allow discovering fully compensated hemolysis and bringing towards a red cell disorder. On the other hand, it also underlines the importance to systematically check for IO all patients with a red cell disorder, who may display high LIC. Our present genotypic data (and previous data) suggest a relative frequency of combined inherited disorders of iron and red cells, making the combined search for both disorders quite relevant in clinical practice. This is now possible with the use of NGS analysis, which allows sequencing large numbers of genes. For those patients with no identified mutation, approaches using whole exome or genome can be proposed as the next step.

P290
CHANGES IN LIVER IRON CONCENTRATION R2 MRI MEASUREMENT ACROSS DIFFERENT CHELATION REGIMENS IN PATIENTS WITH HEMATOLOGICAL DISORDERS: REAL-LIFE EXPERIENCE FROM LICNET

Aims: To assess the impact of different chelation regimens on liver iron concentration (LIC) in patients with hematological disorders.

Methods: A prospective, single-center, non-randomized, non-controlled, observational study was conducted. LIC was measured at baseline and after 12 weeks of treatment with different chelation regimens.

Results: A total of 130 patients were evaluated in this analysis, with a median age of 57 years (range 18-87). LIC was significantly lower in patients receiving DFO monotherapy compared to other regimens (p<0.05).

Summary/Conclusions: This study suggests that different chelation regimens can affect LIC, with DFO monotherapy showing the best results.
THE RELATIONSHIP BETWEEN SERUM FERRITIN AND LIVER IRON CONCENTRATION IN PEDIATRIC CANCER SURVIVORS

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Background: There is increasing recognition that pediatric cancer survivors are at risk of transfusion-related iron overload related to intensive treatment regimes and improved survival rates. Current screening approaches rely on serum ferritin (SF). However, little is known about the SF to liver iron concentration (LIC) relationship in pediatric cancer survivors and whether SF thresholds derived from other iron overload disorders or age groups are appropriate.

Aims: The aim of this study was to investigate the relationship between SF and LIC in pediatric cancer survivors and to determine SF thresholds for predicting clinically significant LICs in this patient group.

Methods: In this retrospective study, patient data were extracted on survivors with elevated ferritin or iron overload from the University of Minnesota Childhood Cancer Survivor Program research database. All patients were enrolled into the database via an informed consent process according to the guidelines of the University of Minnesota Institutional Review Board. Survivors were retrospectively identified once they reached 18 years of age. Seventeen individual survivors were identified where both SF and LIC data were available and the time between the SF and LIC measurement was less than 30 days. Eleven of the 17 survivors had multiple SF measurements producing a final dataset with 34 pairs of SF and LIC measurements. Blood for serum ferritin was collected during clinic visits and analyzed by the University of Minnesota Medical Center, Fairview CLIA-certified clinical laboratory. Liver iron concentration measurements were made using spin density projection-assisted 2D-MRI (FerrScan®).

Linear regression was used to determine the relationship between SF and LIC. Receiver operating characteristic (ROC) curve analysis was used to assess the sensitivity and specificity of SF concentrations for predicting LIC.

Results: The average age of the cohort (6 females and 11 males) at their first SF/LIC measurement was 18.3 years (range 9 to 30.3 years). Acute lymphoblastic leukemia (N=5) and acute myeloid leukemia (N=4) were the most common diagnoses and 15 of the 17 survivors had received a haematopoietic stem cell transplant (HSCT). The average length of time between the final treatment and the first SF/LIC measurement was 5.4 years (range 0 to 12.5 years). A linear fit to all 34 LIC-SF measurement pairs (Figure 1) produced a gradient of 63 ± 15 mg ferritin/L (r²=0.36). The ROC curve analysis (Table 1) indicated that, in this cohort, a SF cut-off of 1270 mg/L potentially has good sensitivity and specificity for predicting a LIC above 15 mg Fe/g and a SF cut-off of 1076 mg/L has poor diagnostic performance for predicting a LIC above 7 mg Fe/g.

Summary/Conclusions: IDA during late pregnancy adversely affects cord blood iron and hearing status. ABR results are closely related the severity of maternal and neonatal iron status. Antenatal screening of pregnant mothers is needed to improve fetal iron status and prevent abnormal auditory maturation.

DECREASED MCP-1 LEVELS IN PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA: A CYTOKINE SIGNATURE OF IRON DEFICIENCY

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Background: Sustained iron deficiency is a major determinant of erythropoietin (Epo) resistance and consequent persistence of anemia in severely affected Hereditary Hemorrhagic Telangiectasia (HHT) patients. MCP-1, identified for predicting clinically important LIC values are considerably lower than observed for thalassemia or adult HSCT patients. This difference in the relationship between SF and LIC for different patient and age groups highlights the difficulty in relying on SF to screen for and define iron overload.

Methods: The study includes 18 HHT patients, 9 males and 9 females, aged 32-79 years, followed at the Hematology Service of CHP-HAS from 2013 to 2017. They all had history of persistent epistaxis (with variable frequency and severity) but without gastrointestinal bleeding. The most severe cases (n=6) were resistant to iron treatment being transfusion dependent. Blood samples were collected in all cases for determination of erythropoietin parameters (including reticulocyte counts, Epo and soluble transferrin receptors (sTfR)) levels iron parameters (transferrin saturation, serum ferritin and hepcidin) and a cytokine panel (IL-6, TNF-α, IL-1β, IL-18, IFN-γ, MCP-1). The same parameters were determined in a group of 16 patients (5 males and 11 females aged 31-81 years) with iron deficiency (ID) due to chronic gastrointestinal bleeding under intravenous iron treatment and in a control group of 21 apparently healthy blood donors (9 males and 12 females aged 38-62 years). Magnetic Resonance Imaging (MRI) was used to assess tissue iron stores in liver, spleen and bone marrow.

Results: Severe anemia with absolute iron deficient (confirmed by appropriate hepcidin downregulation and absence of bone marrow iron stores by MRI) was evident in transfusion dependent HHT patients (TDDHT). Epo resistance in these cases was evidenced by an exponential increase of Epo levels correlated with parameters of severe anemia and ID with highly increased sTfR but inappropriate reticulocyte counts. Significantly decreased MCP-1 levels were observed in TDDHT patients but also in the other iron deficient groups. No significant alterations were observed in other cytokines except for IP-10 which was moderately increased in TDDHT patients. In general, there is a linear decrease of MCP-1 with decreasing Hgb and increasing Epo levels. This effect, however, seems to be “blunted” in severely anemic TDDHT patients with Epo levels above 200 UI/L.

Summary/Conclusions: What is the sensing pathway downregulating MCP-1, and whether an insufficient MCP-1 downregulation contributes to Epo resistance and persistence of severe anemia in TDDHT patients, these are pending questions deserving further investigation.

FERRIC CARBOXYLAMALTOSE VERSUS IRON SUROSE COMPLEX IN WOMEN WITH IRON DEFICIENCY ANEMIA – A RANDOMISED CONTROLLED TRIAL

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Background: Anemia is a condition in which the number of red blood cells or their oxygen-carrying capacity is insufficient to meet physiologic needs, which vary by age, sex, altitude, smoking, and pregnancy status. The WHO Global Database on Anaemia for 1999–2005, covering almost half the world’s population, estimated the prevalence of anaemia worldwide at 25 percent. India falls in the ‘severe’ category of public health significance. Ferric carboxymaltose (FCM) comprises of a macromolecular iron hydroxide complex of polynuclear Fe3+ hydroxide tightly bound in a carbohydrate shell. The molecular structure of ferric carboxymaltose ensures controlled delivery of iron within cells of reticuloendothelial system and subsequent delivery to the iron binding proteins ferrochelatase.
P295

GENOME-WIDE ASSOCIATION STUDY OF HODGKIN LYMPHOMA IDENTIFIES HISTOLOGY-SPECIFIC ASSOCIATIONS AND TRANSCRIPTIONAL REGULATORS OF DISEASE SUSCEPTIBILITY

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Background: Several susceptibility loci for Hodgkin lymphoma (HL) have been reported, however much of the heritable risk and biological relevance remains unknown.

Aims: To identify novel risk loci for HL and histological subtypes and to further our understanding of how genetic risk loci influence disease susceptibility.

Methods: To our knowledge, we have performed the largest genome-wide association study of HL totalling 5,156 cases and 16,763 controls across 10 million single nucleotide polymorphisms. We have integrated gene expression, chromatin state, transcription factor (TF) binding and capture Hi-C in model B-cells to functionally annotate new and existing risk loci.

Results: We identified risk loci for all HL at 6q22 (rs9482849, PTPRK, P = 1.52 × 10^-10) and for nodular sclerosis HL (NSHL) at 3q28 (rs4459895, LPP, P = 9.49 × 10^-13), 5q23 (rs6928977, AHIT, P = 4.62 × 10^-10), 10p14 (rs3781093, GATA3, P = 9.49 × 10^-13), 13q34 (rs112988813, UFPP3, P = 4.58 × 10^-8) and 16p13 (rs34972832, CLEC16A, P = 1.29 × 10^-8). Additionally, independent loci within the HLA region were observed for NSHL (rs2698014, HLA-DPB1*03:01, Val86 in HLA-DRB1) and mixed cellularity HL (rs163396, rs13196329, Val86 in HLA-DRB1). Expression quantitative trait loci were observed in lymphoblastoid cells from 825 individuals at 6q23 (AHIT, PSMR=8.63x10^-6) and 10p14 (GATA3, PSMR=4.70x10^-6). Across new and established risk loci we confirmed a significant enrichment of DNase hypersensitivity in GM12878 cells (P = 1.20 × 10^-10), as well as regulatory elements in primary B-cells (P = 6.0 x 10^-6) and in macrophages (P = 6.85 × 10^-5). Analysis of ChIP-seq data on 82 transcription factors (TFs) in GM12878 cells, showed an over-representation of the binding of TFs that play a central role in B-cell signalling-networks such as RELA (nuclear factor NF-kappa-B p65), EBFB1 (early B-cell factor 1), RUNX3 (run-related transcription factor 3) and BATF (basic leucine zipper transcription factor, ATF-like).

Summary/Conclusions: These observations support the assertion that risk loci for HL mediate their effects through B-cell developmental networks, and are involved in transcriptional initiation and enhancement. Furthermore, our findings emphasise the differences between the major subtypes, which are likely reflective of differences in disease aetiology.

P296

SOX11 PROMOTES TUMOR PROTECTIVE MICROENVIRONMENT INTERACTIONS IN MANTLE CELL LYMPHOMA

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Background: Mantle Cell lymphoma (MCL) is one of the most aggressive...
phoid neoplasms characterized by highly infiltrated tumor cells in lymphoid tissues and extra nodal sites. The patients have short responses to current therapies and frequent relapses. However, recent studies have identified a subset of MCL with indolent clinical behavior that tends to present with leukemic disease instead of extensive nodal infiltration, and that is characterized by the absence of the transcription factor SOX11 [SRY (Sex determining region-Y) box 11]. SOX11 oncogetic pathways drive MCL tumor progression are poorly known.

**Aims:** The goal of our study was to identify the spectrum of genes regulated by SOX11 in malignant lymphoid cells and provide insights on how the constitutive overexpression of SOX11 may contribute to the oncogenic development of MCL.

**Methods:** We utilized immortalized intertumoral subsets of DLBCL cells with reduced SOX11 protein levels by infecting MCL cell lines with lentiviral particles carrying shRNA plasmids specifically targeting SOX11. SOX11-positive MCL cell line was infected with the empty vector and used as a control. These two MCL cell lines were injected in two different mice models to analyze in vivo the role of SOX11 in DLBCL, subgingival (sc) and peritoneal (sp) xenograft tumor models. To analyze the crosstalk between MCL and microenvironment, we did in vitro cocultures experiments using accessory cells at the tumor microenvironment, as endothelial and bone marrow mesenchymal cells.

**Results:** In the sc mice model, we observed that SOX11 silencing reduced tumor growth compared to SOX11-positive control tumors. We analyzed the gene expression profiling of these xenograft tumors and of SOX11-positive and negative primary cases and we observed that different microenvironment-related signatures were enriched in SOX11-positive compared with SOX11-negative cells, as angiogenesis, migration and stromal stimulation. By ChIP-chip studies, we found that SOX11 regulates expression of VavP-Bcl2 in tumor cells (64,323 AICDA-perturbed CpGs), suggesting a conserved epigenetic function of AICDA in GC B cells and human and mouse GC-derived lymphomas. Finally, we found significant overlap between genes affected by AICDA-perturbed CpGs in human AICDA-high DLBCLs and murine VavP-Bcl2 AICDA lymphomas (P=2.21e-23) and with the genes affected by AICDA in GC B-cells (P=8.48e-33).

**Summary/Conclusions:** Our results demonstrate that AICDA acts as a methylome modifier in GC-derived lymphomas, introducing epigenetic heterogeneity, which may provide tumor cells with higher capacity to adapt to an evolving microenvironment. These findings are relevant not only for B-cell lymphomas, but also for other types of cancer expressing cytosine deaminases.

**P298**

**XP0 INHIBITION SYNERGIZES WITH BCR INHIBITION, BLOCKS TUMOR GROWTH AND PROLONGS SURVIVAL IN A BIOLUMINESCENT ANIMAL MODEL OF PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA**

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**Aims:** We here report the results of a phase I study designed to evaluate the safety, tolerability and antileukemic activity of XP0 (GROWTH AND PROLONGS SURVIVAL IN A BIOLUMINESCENT ANIMAL MODEL OF PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA)**

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**Background:** Primary central nervous system lymphoma (PCNSL) is an non-Hodgkin lymphoma localized in the CNS. Approximately 95% of PCNSL are classified as diffuse large B-cell lymphoma (DLBCL), being most of them related to activated B-cell type (ABC-DLBCL). PCNSL is associated with poor prognosis, particularly because of the difficulty for drugs to cross the blood brain barrier. High dose methotrexate is the most effective treatment, but relapse is very common and salvage treatment options are scarce. Also, in patients with systemic lymphoma, the secondary infiltration of the CNS is a fatal event, with a global overall survival of less than six months. Therefore, the development of new drugs with ability to penetrate the CNS is highly needed. Selinexor (KPT-330) is a Selective Inhibitor of Nuclear Export (SINE) that inactivates XPO-1 protein and induces anti-tumor effects mainly due to forcing nuclear retention and activation of tumor suppressors. Selinexor has shown excellent brain penetration and promising results in pre-clinical models of glioblastoma and can inhibit both BCR and NF-kB signaling in malignant B-cells.

**Aims:** In order to provide a pre-clinical rationale for the design of new therapies for patients with CNS lymphoma our main aim is to assess the role of XPO-1 inhibition in intracerebral xenograft murine models.

**Methods:** We in vitro tested the sensitivity of DLBCL cell lines to selinexor andibrutinib by MTS and AnnexinV/PI assay. We established an orthotopic xenograft model of PCNSL by stereotactic injection of OCI-Ly10 (ABC, MYD88 and CD79b mut) cells expressing luciferase into the cerebral parenchyma of SCID mice to ibrutinib and showed strong synergism between the two compounds. To analyze the role of XPO-1 we infected MCL cell line was infected with the empty vector and used as a control. These xenograft tumors and of SOX11-positive and negative primary cases and we observed that different microenvironment-related signatures were enriched in SOX11-positive compared with SOX11-negative cells, as angiogenesis, migration and stromal stimulation. By ChIP-chip studies, we found that SOX11 regulates expression of VavP-Bcl2 in tumor cells (64,323 AICDA-perturbed CpGs), suggesting a conserved epigenetic function of AICDA in GC B cells and human and mouse GC-derived lymphomas. Finally, we found significant overlap between genes affected by AICDA-perturbed CpGs in human AICDA-high DLBCLs and murine VavP-Bcl2 AICDA lymphomas (P=2.21e-23) and with the genes affected by AICDA in GC B-cells (P=8.48e-33).

**Summary/Conclusions:** Our results demonstrate that AICDA acts as a methylome modifier in GC-derived lymphomas, introducing epigenetic heterogeneity, which may provide tumor cells with higher capacity to adapt to an evolving microenvironment. These findings are relevant not only for B-cell lymphomas, but also for other types of cancer expressing cytosine deaminases.

**Results:** In the sc mice model, we observed that SOX11 silencing reduced tumor growth compared to SOX11-positive control tumors. We analyzed the gene expression profiling of these xenograft tumors and of SOX11-positive and negative primary cases and we observed that different microenvironment-related signatures were enriched in SOX11-positive compared with SOX11-negative cells, as angiogenesis, migration and stromal stimulation. By ChIP-chip studies, we found that SOX11 regulates expression of VavP-Bcl2 in tumor cells (64,323 AICDA-perturbed CpGs), suggesting a conserved epigenetic function of AICDA in GC B cells and human and mouse GC-derived lymphomas. Finally, we found significant overlap between genes affected by AICDA-perturbed CpGs in human AICDA-high DLBCLs and murine VavP-Bcl2 AICDA lymphomas (P=2.21e-23) and with the genes affected by AICDA in GC B-cells (P=8.48e-33).

**Summary/Conclusions:** Our results demonstrate that AICDA acts as a methylome modifier in GC-derived lymphomas, introducing epigenetic heterogeneity, which may provide tumor cells with higher capacity to adapt to an evolving microenvironment. These findings are relevant not only for B-cell lymphomas, but also for other types of cancer expressing cytosine deaminases.
was measured and animals were randomly distributed into drug or vehicle group. At this time point mice were treated with 5mg/kg of selinexor or vehicle via oral gavage three times a week; subsequently, bioluminescence was assessed twice a week. Treatment with selinexor significantly increased mice survival, with a median survival of 48 days in the treatment group compared to 34 days in the vehicle group (p<0.0001; Figure 1A). Mice in the treatment group also lived a length of time significantly longer in comparison with the control group (ANOVA: p<0.0001; Figure 1B). Specific time-point analysis showed that differences were significant as soon as 8 days after treatment. At final point, histopathological analysis showed diffuse infiltration in meninges and cerebral parenchyma of highly proliferative CD20-positive B-cells. Currently, we are evaluating the synergy between ibrutinib and selinexor in vivo. For that we have used the same experimental setting and assigned 12 mice to each of the following groups: selinexor only (5mg/kg three times a week via oral gavage), ibrutinib only (25mg/kg daily in drinking water), combination or vehicle. Results will be available at the time of the meeting.

Figure 1.

Summary/Conclusions: Selinexor inhibits proliferation and survival of DLBCL cell lines regardless of COO and it can synergize with ibrutinib. Treatment of mice with CNS confined ABC-DLBCL with selinexor significantly reduces tumor growth and increases survival. Our results provide preclinical evidence for the development of selinexor as new therapeutic option for PCNSL or DLBCL with CNS involvement.

P299

MOLECULAR HETEROGENEITY IN PERIPHERAL T-CELL LYMPHOMA NOT OTHERWISE SPECIFIED REVEALED BY COMPREHENSIVE MUTATIONAL PROFILING


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Background: Peripheral T-cell lymphomas (PTCLs) are a highly heterogeneous group of mature T-cell neoplasms. In particular, accounting for the majority of PTCL, PTCL not otherwise specified (PTCL-NOS) is a diagnosis of exclusion and for such, is suspected to include many heterogeneous tumors. In fact, recent genetic studies have suggested that a subset of PTCL-NOS is closely related to angioimmunoblastic T-cell lymphoma (AITL); both lymphoma types show follicular helper T-cell (TFH) phenotypes and share mutational targets in common, such as RHOA, TET2, DNMT3A, and IDH2. However, with the lack of comprehensive genetic analyses, the molecular pathogenesis is poorly understood in the majority of PTCL-NOS cases.

Aims: The aim of this study is to clarify a landscape of somatic mutations in PTCL-NOS.

Methods: We performed whole-genome/exome and transcriptome sequencing of PTCL-NOS and other related PTCLs, followed by targeted-capture sequencing of candidate drivers in T-cell lymphomas in 100 PTCL-NOS samples.

Results: Consistent with previous reports, TET2 (38%) was the most frequently mutated gene in PTCL-NOS, followed by RHOA (28%), TP53 (18%), KMT2C (13%), IDH2 (11%), and PLCG1 (11%). Frequently altered genes included signal transduction molecules such as RHOA, PLCG1, STAT3 and SOCS1, chemokine receptors (CCR4 and CCR7), epigenetic modifiers (TET2, KMT2C, IDH2, DNMT3A, CREBBP, and KDM6A), and molecules associated with immune evasion (HLA-A, HLA-B, B2M, and CD58). Novel targets of recurrent mutation were also identified, including PDCD1, YTHDF2, and LRPIB, which were frequently targeted by nonsense and frameshift mutations distributed throughout the entire genes. Among these, PDCD1 encodes PD-1, which transmits an inhibitory signal from PD-L1 and PD-L2 ligands, and therefore loss of function of this gene is predicted to enable malignant T-cells to escape from the negative signaling. By contrast, recurrent mutations in YTHDF2 and LRPIB mutations in T-cell lymphomagenesis is unexpected. These genes encode a repressor protein of N6-methyladenosine (YTHDF2), and a member of the low density lipoprotein receptor family (LRPIB). Although the function of these genes in T-cells are unknown, our findings suggest their unresolved roles, whose dysfunction may lead to malignant T-cell proliferation.Finally, we investigated the co-occurrence between frequently mutated genes in PTCL-NOS. In accordance with previous observation, mutations characteristic of TFH lymphomas (TET2, RHOA, IDH2, and DNMT3A) tended to co-occur in a subset of PTCL-NOS cases, but were almost mutually exclusive with mutations in TP53 and chemokine receptor genes. These observations further support the molecular distinction between TFH and non-TFH lymphomas in PTCL-NOS: the former is more related to AITL and discriminated from the latter in terms of their mutational profiles.

Summary/Conclusions: In summary, our findings illustrate the landscape of somatic alterations in PTCL-NOS and provide a novel insight into their genetic and molecular heterogeneity, which should help to devise a novel molecular classification of PTCLs and to exploit a new therapeutic strategy to combat these intractable T-cell malignancies.

P300

A COMPREHENSIVE PORTRAIT OF THE DNA METHYLMOE OF 866 SAMPLES FROM DIFFERENT B CELL NEOPLASMS: BIOLOGICAL INSIGHTS AND CLINICAL APPLICATIONS


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Background: In the last years, a large body of evidence has been accumulated demonstrating that DNA methylation is not only widely altered in B-cell lymphoid tumors (and cancer in general) but it is also defining cell lineage and maturation stage. However, an integrative study of the whole DNA methylation of neoplastic B cells from different maturation stages has not been performed yet.

Aims: The aim of this study was to extensively dissect the dynamics of DNA methylation in B-cell neoplasias in the light of normal B cell maturation program. The ultimate goal of this study was to generate new clinically relevant knowledge with diagnostic and prognostic value.

Methods: Our dataset included whole-genome bisulfite sequencing data (n=57) and high-density methylation arrays (n=1161) from acute lymphoblastic leukemia (ALL), mantle cell lymphoma (MCL), Burkitt lymphoma (BL), follicular lymphoma (FL), diffuse large B cell lymphoma (DLBCL), chronic lymphocytic leukemia (CLL) and multiple myeloma (MM) patients as well as from ten different normal B cell subpopulations. As DNA methylation estimates in neoplastic samples are influenced both by tumor cell content and composition of the micro environment, we developed a new method to deconvolute and in silico purify the methylation signal of tumors arising in different niches (bone marrow, peripheral blood an lymph node). The data were analyzed by a series of bioinformatic and biostatistical approaches and correlated with clinical variables.

Results: The initial bioinformatic approach to purify DNA methylation signals in B cell tumors revealed that samples with less than 55% tumor cell content could not be accurately purified. This strategy reduced the initial 1,044 tumor samples to 866. An unsupervised principal component analysis of in silico purified data revealed that each type of B-cell neoplasm clusters separately. ALLs clustered closer to precursor B cells, CLL and MCL closer to mature B cells and both DLBCL and MM showed the largest deviation from normal B cells. We then performed a differential methylation analysis in tumor samples vs normal B cell maturation stages, and thoroughly annotated the results to biological and clinical features. From the clinical perspective, we identified that for tumor samples with similar cellular origin, the higher the epigenetic deviation from healthy B cells (number of DNA methylation changes) the worse the clinical outcome of the patients. Furthermore, for each tumor entity, we could identify from 5 to 19 epigenetic biomarkers that could classify each entity with high sensitivity and specificity.

Summary/Conclusions: In this study, we show that in silico purification of DNA methylation data is a powerful strategy to accurately measure DNA methylation alterations in tumor cells. Using a large dataset, we have developed a set of epigenetic biomarkers with high differential diagnostic power and identified that the epigenetic drift is a universal prognostic factor that can be applied to different B cell tumors.
ACTIVATION OF RHOA-VAV1 SIGNALING AXIS IN ANGOIMMUNOBLASTIC T-CELL LYMPHOMA

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Background: Angioimmunoblastic T-cell lymphoma (AITL) is a distinct subset of peripheral T-cell lymphoma with follicular helper T-cell (TFH) features. We and others previously found mutations of RHOA, encoding p.Gly17Val (G17V) RHOA (RHOA) mutants, in AITL patients in comparison with those in normal mononuclear cells derived from peripheral blood mononuclear cells (PBMCs). G17V RHOA is a small GTPase, is converted from the GDP-bound inactive state to the active GTP-bound form by guanine nucleotide exchange factors (GEFs). The G17V RHOA mutant has been shown to be defective in RHOA dependent and –independent function. VAV1 activation is tightly regulated by specific binding partner proteins of the G17V RHOA mutant were examined by high throughput screening in Jurkat cells. Nuclear factor of activated T cell (NFAT) activity in response to TCR stimulation was examined in Jurkat cells expressing wild-type (WT) and G17V RHOA mutant, and WT and various VAV1 mutants. Whole transcriptome was compared in Jurkat cells inducibly expressing each cDNA, in conditions with or without TCR stimulation. Expression of phospho-Vav1 was examined by immunostaining for AITL/TFF lymphoma samples.

Methods: Proteomic screening was performed to identify G17V RHOA-specific binding partner proteins. Binding was validated by co-immunoprecipitation of G17V RHOA and the candidate partners. Simultaneously, RNA sequencing was performed for 9 PTCL samples, including 6 AITL and 3 PTCL-NOS. Targeted deep sequencing of VAV1 was performed for 126 PTCL samples, including 69 AITL and 57 PTCL-NOS, 37 of which had RHOA mutations. The specific binding partner proteins of the G17V RHOA mutant were examined by high throughput screening in Jurkat cells. Nuclear factor of activated T cell (NFAT) activity in response to TCR stimulation was examined in Jurkat cells expressing wild-type (WT) and G17V RHOA mutant, and WT and various VAV1 mutants. Whole transcriptome was compared in Jurkat cells inducibly expressing each cDNA, in conditions with or without TCR stimulation. Expression of phospho-Vav1 was examined by immunostaining for AITL/TFF lymphoma samples.

Results: Proteomic screening identified the VAV1 protein as a G17V RHOA-binding partner. RNA sequencing identified the VAV1 protein as a G17V RHOA-binding partner. Phosphorylation was blocked by the dasatinib in Jurkat cells expressing the G17V RHOA or VAV1-STAP2 cDNA compared to those expressing each WT cDNA or mock. Phosphorylation was blocked by the dasatinib in Jurkat cells expressing the G17V RHOA or VAV1-STAP2 cDNA than those expressing each WT cDNA or mock. Gene set enrichment analysis showed that cytokine and chemokine-related pathways were enriched in Jurkat cells expressing the G17V RHOA compared to those with WT or mock. Finally, phospho-VAV1 was co-stained with PD-1, a TFH marker, in 7 out of 10 PTCL samples with RHOA or VAV1 mutations.

Summary/Conclusions: The G17V RHOA and VAV1 mutants both intensify the TCR pathway through hyper-phosphorylation of Vav1. Our data suggest that the RHOA-VAV1 axis in AITL/TFF lymphoma may contribute to their clinical features and stand as a possible new therapeutic target.
formation using a bespoke bioinformatic pipeline based on TargetScan prediction algorithm in order to identify mutations in putative miRNA binding sites. Once identified, in order to validate them and test their recurrence in an extended cohort (60 samples from 31 FL patients who underwent transformation plus 21 samples of non-transformed FL patients) we designed an Ampliseq (Ion Torrent, Life Technologies) NGS custom panel. Finally, we selected a number of variants for assessing the variant effect on the miRNA:miRNA interaction, by means of a combination of an in silico predictive algorithm and in vitro luciferase assays.

**Results:** 36% of somatic variants from WGS data arose in 3’UTR, and 68% of these were putative miRNA-binding sites (525 mutations in 497 genes). Interestingly, the ontology analysis showed that these mutations were not randomly distributed but rather there was enrichment in genes associated with haematological malignances (P=2.18x10^-4). We then validated 85% of these mutations using targeted resequencing and found a total of 103 recurrent variants located in putative miRNA binding sites. QC criteria filtering led us to prioritise 38 variants in 25 genes to be functionally tested. Crucially, ontology analysis showed that these genes were highly enriched for GC-like B-cell lymphoma genes (P=4.39x10^-5), strongly suggesting that these variants may have a biological significance in the disease. We then performed an in silico approach based on TargetScan miRNA target prediction algorithm to evaluate the effect of the mutations on the binding of the miRNAs to their target sites. Based on these results we prioritized some of these genes to perform luciferase assays. We experimentally demonstrated not only that the majority of these loci are bona fide miRNA targets sites, but also that the presence of a number of these variants cause a dysregulation of the normal miRNA regulatory activity (Figure 1).

**Summary/Conclusions:** Our data show that the identified mutations do not occur randomly, but preferentially in putative microRNA binding sites of genes related to lymphomagenesis, supporting their role in FL pathogenesis. Furthermore, the presence of some of the identified variants in miRNA binding sites indeed promotes a dysregulation of the normal miRNA regulatory activity, suggesting that they might have a biological significance in FL.

**P304**

**CLINICAL IMPACT OF TP53 AND KMT2D MUTATIONS IN MCL RECEIVING HIGH-DOSE THERAPY AND AUTOLOGOUS TRANSPLANTATION: UPDATED RESULTS FROM THE FONDAZIONE ITALIANA LINFOMI MCL0208 PHASE III TRIAL**

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**Background:** Within the landscape of mutated genes in mantle cell lymphoma (MCL), only TP53 disruption has been so far associated with outcome.

**Aims:** Here we present the clinical update of the deep sequencing MCL gene panel analysis in the prospective FIL-MCL0208 phase III trial (NCT02354313), high-dose immunochemotherapy followed by autologous transplantation for untreated, advanced stage <65 years MCL) based on the data from the second interim analysis.

**Methods:** A targeted resequencing gene panel, including coding exons and splice sites of the ATM, BIRC3, CCND1, KMT2D, TP53, TRAF2, WHSC1, and NOTCH1 genes was analyzed in tumor DNA from baseline bone marrow CD19+ purified MCL cells and, to filter out polymorphisms, in the paired normal genomic DNA (55% of cases) using a TrueSeq Custom Amplicon target enrichment system followed by deep next generation sequencing (Illumina, median depth of coverage 2356x). Variants represented in >10% of the alleles were called with VarScan2 with the somatic function when the paired germline DNA was available. For patients lacking germline DNA, a bioinformatics pipeline including a number of stringent filters was applied to prevent against the misclassification of polymorphisms as somatic variants. Clinical data were updated at the time of the second interim analysis (January, 2017).

**Results:** Out of the 300 enrolled patients, 174 were evaluable for mutations. Median follow-up of the cohort was 36 months, and 3-years PFS and OS were 67% and 86%, respectively. Patients not included in the study, due to unavailable tumor DNA (n=126) showed superimposable clinical features and outcome. Mutations of TP53 (8% of cases) and KMT2D (11% of cases) were associated with an increase in the hazard of progression both in univariate analysis as well as after adjusting for MIPI, Ki67 and blastoid variant: HR 3.87 (95% CI 1.64 to 9.13), p=0.002 and HR 3.66 (95% CI 1.77 to 7.56), p=0.001, respectively. These results translated into an increase of the hazard of death in both TP53 and KMT2D mutated patients both in univariate analysis as well as adjusting for MIPI, Ki67 and blastoid variant: HR 4.26 (95% CI 1.34 to 13.57), p=0.014 and HR 3.09 (95% CI 1.07 to 8.86), p=0.036, respectively. On these bases, a survival model was proposed based on the TP53 and KMT2D mutation status: 3-years PFS and OS were 26% and 64% for patients carrying either TP53 or KMT2D mutations or both vs 75% and 92% for patients without any of these mutations (Figure 1).

**Summary/Conclusions:** The updated clinical results of the FIL-MCL0208 trial show that: i) both TP53 and KMT2D mutations independently associate with shorter PFS and OS in younger MCL patients receiving high-dose therapy; ii) KMT2D mutations seem to be as detrimental as TP53 mutations, at least in terms of PFS; iii) given the negative prognostic impact of these mutations, they might be used to select high-risk patients for novel therapeutic approaches.
Multifaced aspects of bleeding disorders

P305
A LOOKBACK AT VWD TYPE 2A AND 2M CLASSIFICATION IN A LARGE COMPREHENSIVE HAEMOPHILIA CENTRE.
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Background: Von Willebrand Disorder (VWD) has a prevalence of approximately 1% in the general population and is due to quantitative deficiencies or qualitative defects of the Von Willebrand Factor (VWF) protein. VWF is a large multimeric protein with multiple functions. It carries and protects factor VIII and helps in the binding of FVIII, platelets and the vascular endothelium at sites of injury. VWF binding to platelets is through several receptors notably the glycoprotein 1b (GP1b) and collagen exposed at site of injury is important for VWF adhesion to the subendothelial matrix forming an adhesive anchor. Classification of VWD is based on the quantitative deficiencies (Type 1 and 3) and VWD type 2 are qualitative defects of the VWF protein with or without quantitative deficiency as well. Type 2 VWD is further subdivided into type 2A,2B,2M and 2N. These subtypes depend on a number of laboratory assays that measure the FVIII activity, VWF protein level (VWF:Ag) and the function of the protein i.e its ability to bind to 1) FVIII-VWF binding assays (2) platelets (VWF Rcof assay) and 3) collagen (VWF:CB assay). Other tests include ristocetin induced platelet aggregation (RIPA), multimer analysis, assay ratios and VWF genetic analysis. No single commercially available laboratory method can achieve to test all the parameters required to clinch the accurate diagnosis of the subtypes of VWD. Use of these triple assays with VWF Rcof/FVIIIc/FVIII:Ag ratio, VWF CB (VWF-CB) (VWF Ag) ratio have helped in the better identification of VWD and the subtypes.

Aims: To assess recent various VWF investigation panels and assay ratios, VWF genetic analysis, multimeric patterns of the VWF protein in accurate diagnosis of the VWD subtypes. VWD 2A and 2M shows similarities in certain aspects and it is important to differentiate these 2 subtypes as new therapies become available and personalised treatment approaches of VWD become a reality.

Methods: Clinicians who have made a diagnosis of VWD for individuals referred for a bleeding state work up would classify the subtypes of the VWD according to the results of the investigations available at the time of seeing the patients. All patients with an inherited bleeding disorder would then be registered in the centre and details would be put into a database. We have looked back into the database from the period of 2000 to end of 2016 and focussed on the VWD types 2A and 2M. Current VWD diagnostic panel in our centre includes the following tests: FVIII one stage assay, VWF:Ag Elisa,VWF ristocetin, Platelet agglutination method, VWF CB Elisa methods, VWF multimeric analysis by gel chromatography and VWF exon 27/28 genetic mutations are routinely done. New information and new set of results for the registered patients have been taken into account the classification of VWD type 2A and 2M and the database are updated.

Results: In the VWD database 36 patients classified as 2M and 19 patients as type2A have been recorded from 2000 to end of 2016. With the updated results and genetic analysis and the response to DDAVP, around 30% of the patients have had their subtypes changed. This exercise confirms that no singular test can be used to accurately diagnose the VWD and its subtypes and illustrates the importance of DDAVP testing and the difficulty of interpreting assay ratios for the VWD subtypes. Existing assay levels are <15%.

Summary/Conclusions: VWD may be misdiagnosed, underdiagnosed or overlooked. Appropriate and complete investigative panel is necessary for complete classification of VWD and its subtypes.

P306
RETROSPECTIVE EVALUATION OF PHENOTYPE AND MANAGEMENT OF DYSFIBRINOGENEMIA AND HYPODYSFIBRINOGENEMIA IN A COHORT OF ITALIAN PATIENTS
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Background: Dysfibrinogenemia (DF) and hypofibrinogenemia (HDF) patients (pts) experience hemorrhages or thromboses, and the clinical management can be difficult.

Aims: Aim of this study is to obtain information on DF/HDF clinical phenotype and management.

Methods: This is a spontaneous, retrospective, multicenter national study. Data is collected from clinical records.

Results: Forty-one pts have been enrolled in 3 centers: 35 DF (85%), 6 HDF (15%); 18M, 23F. Median follow-up: 7.4 months (1-203). Median age at diagnosis: 36 years (range 3-81); Median fibronogen activity/antigen level: 53 mg/dl (0-156) and 250 mg/dl (66-380), respectively. Fourteen pts experienced hemorrhagic events: epistaxis, hematuria, haemophilia, gastrointestinal bleeding, thrombosis, menorrhagia, and gastro-intestinal (presence of esophageal varices). No specific therapy was administered. A portal venous thrombosis occurred in 1 DF splenectomized patient in absence of replacement therapy; he was treated with warfarin without anti-hemorrhagic prophylaxis. Forty-one minor/major surgeries were performed in 23 pts. In 10/41 (24%) cases, prophylaxis was administered (fresh frozen plasma in 3, fibrinogen concentrate (FC) in 1, tranexamic acid in 6); in 5/41 (12%) cases, low molecular weight heparin (LMWH) was administered; no hemorrhage occurred. Thirteen pregnancies were initiated in 9 women. In 1 case, LMWH prophylaxis was administered during pregnancy, and in 1 other during puerperium. In 2 cases, FC was administrated at the time of spontaneous delivery (SD). Nine SD and 4 cesarian sections were performed without complications.

Summary/Conclusions: Pts from this case series experienced few hemorrhagic/thrombotic events. The majority was asymptomatic and the most severe events were related to concomitant pathologies. Nonetheless, this study has the potential to collect data from a numerous population of pts who live in the same country, and therefore to provide useful information to better characterize and manage these rare diseases.

P307
OSTEOPOROSIS IN PATIENTS WITH HEMOPHILIA
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Background: Osteoporosis is often a co-morbidity of hemophilia, which exacerbates the hemophilic arthropathy and affects the long-term stability of the components after the arthroplasty. We present our results for the prevalence of osteoporosis in 148 patients with haemophilia and hemophilic arthropathy.

Aims: To present progression of hemophilic arthropathy and increase the long-term stability of the arthroplasty.

Methods: In the period from 2015 to 2016, the presence of osteoporosis surveyed 148 patients with haemophilia who were hospitalized in the department of reconstructive orthopedics for patients with hemophilia (Moscow, Russia): 121 (81.8%) - hemophilia A, 21 (14.2%) - and hemophilia B 6 (4%) - haemophilia with inhibitor. The average age of the patients was 39.3 years (range 10 to 69 years). 121 patients with hemophilic arthropathy performed primary total arthroplasty (98 knee, 20 hip, 3 shoulder joints); 18 patients underwent revision arthroplasty (5 - purulent infection, 7 - instability of the implants, 4 - fractures, 2 - loss of motion in the operated joint). 40 patients underwent ultrasound densitometry. Ultrasound densitometry was performed in 148 patients. As a result of ultrasound densitometry in 17.5% (7 patients) of cases revealed osteopenia and 20% (8) T-highest index. 105 patients underwent histological study in which 93 (88.6%) bone resorption, 58 (55.2%) intraosseous hemorrhage which 53 (50.5%) cases were accompanied by bone resorption. In total (histologically and of ultrasound densitometry) 99(66.9%)  patients with hemophilia had osteopenia.

Summary/Conclusions: The data indicate that osteoporosis at patients with haemophilia considerably more common than in the general population. Intraosseous hemorrhage identified in more than half of the cases, exacerbate the decline in bone mineral density.

P308
PREVALENCE OF GENETIC MARKERS OF OXIDATIVE STRESS IN PATIENTS WITH SEVERE HEMOPHILIA FROM NORTH-WESTERN RUSSIA
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Background: Severe haemophilia (SH) is often complicated by chronic arthropathy due to recurrent haemorrhagic events and activation of such biological mechanisms as oxidative stress (OS) and inflammation. We have previously shown that the biochemical markers of OS and/or deficiency of antioxidant system (AOS) are frequently seen in SH patients affected with joint(s) destruction. Until now, there is a little data on the frequency of genetic variants predisposing to OS or decreased AOS activity in patients with SH.

Aims: To assess the prevalence of several genetic variants predisposing to OS or decreased AOS activity in patients with SH.

Methods: We studied 71 men with severe haemophilia A or B (62 and 9 patients, respectively). Osteoarthritis of large joint(s) was detected in each
patient, with the rate of recurrent haemorrhagic events in joint(s) from 6 to 13 per year. The control group consisted of 255 age-matched healthy men. Gene polymorphisms of apolipoprotein E (ApoE e2/e3/e4), paraoxonase (PON1 Gln192Arg), methylenetetrahydrofolate reductase (MTHFR C677T), catalase (CAT C-262T) and plasmatic glutathione peroxidase (GPX3 T-165C) was studied by PCR-RFLP technique. Statistical differences between the patient and control group were assessed by Fisher’s exact test. Odds ratios (OR) with their 95% confidence intervals (CI) and p-value were calculated by using GraphPad Prism 5.0 software.

Results: We found abnormal distribution of ApoE genotypes in the patient group. Absence of ApoE e3 allele was observed in 7 (9.9%) men with SH 9 and 8 (3.1%) controls (OR=3.4, 95% Cl. 1.2-9.7, p=0.025). In particular, the frequency of ApoE e2/e2 genotype was 10-fold increased in patients when compared to healthy men (4.2% vs 0.4%, OR=11.2, 95% Cl: 1.1-109.5, p=0.034). ApoE e2/e4 and e4/e4 genotypes were also more prevalent in SH than in the control group (2.8% vs 0.8% and 2.8% vs 0.2%, respectively). In the patient group, we observed the positive association between the PON1 192Gln/Gln variant and heterozygous GPX3 -65TC genotype (OR=5.8, 95% Cl: 1.3-25.7, p=0.021). Simultaneous presence of these genotypes was more than 5-fold found in SH than in controls (8.5% vs 1.6%, 95% Cl: 1.3-22.8, p=0.016).

Summary/Conclusions: Our results indicate that OS-provoking variants of ApoE, PON1 and GPX3 genes are frequently seen in SH patients with chronic arthropathy and joint(s) destruction.

P309
THE ROLE OF DNA METHYLATION AND EXPRESSION OF MMP-2 AND MMP-9 IN PATHOGENESIS OF INTRACEREBRAL HEMORRHAGE IN CONGENITAL FACTOR XIII DEFICIENCY
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Background: Congenital factor XIII deficiency (CFXIIID) is a rare bleeding disorder. Intracerebral hemorrhage (ICH) is a leading cause of mortality and morbidity in this disorder. Matrix metalloproteinase-2 (MMP-2) and MMP-9 are reported to be associated with ICH.

Aims: The purpose of this study was to investigate the association of MMP-2 and MMP-9 methylation and their expression with ICH.

Methods: Patients with abnormal clot solubility test as well as a positive history of FXIII deficiency were participated in the study. Methylation status was analyzed by Bisulfite Sequencing PCR. Gene expression in mRNA and protein levels was assayed by Quantitative real-time RT-PCR and ELISA, respectively.

Results: We found an unmethylated profile for both MMP-2 and MMP-9 in patients with ICH. Both of these genes were partially methylated in controls. Percent of methylated CGs are also higher for MMP-9 than MMP-2. Expression of MMP-9 in both of mRNA and protein levels was found in ICH compared to non-ICH group. However, there were no significant differences in MMP-2 expression (neither mRNA nor protein) between two groups.

Summary/Conclusions: Our findings showed that gene methylation contributes effectively in regulation of MMP-9 expression. Furthermore, our data suggest that MMP-2 expression in CFXIIID may not be controlled by gene methylation alone because methylation status of this gene did not correlate with expression levels (neither mRNA nor protein). Further investigations are needed for better understanding the exact role of these MMPs in the pathogenesis of ICH in CFXIIID and also identifying the regulatory mechanisms.

P310
GENETIC CONFIRMATION AND FINDING NOVEL MUTATIONS IN GLANZMANN THROMBOASTHENIA AND VON WILLEBRAND DISEASE FAMILIES BY DIAGNOSTIC EXOME SEQUENCING
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Background: Congenital platelet function disorders and von Willebrand disease (vWD) are very heterogeneous group resulting in primary hemostatic defects. Physicians generally have difficulty to confirm them due to complicated diagnosis.

Aims: We intended to apply diagnostic exome sequencing (DES) for genetic confirmation and finding causative variants in children with primary hemostatic problems.

Methods: Library preparation was performed with TruSight One sequencing panel (Illumina, USA), which enriches about 4,800 genes with clinical relevance. Massively parallel sequencing was conducted with NextSeq (Illumina). Variants were annotated with population databases (1000 Genomes Project, Exome Variant Server, Exome Aggregation Consortium) and disease databases (OMIM). For missense variant, in-silico analysis was done with SIFT, PolyPhen-2, and MutationTaster. Candidate variants were confirmed by Sanger sequencing and family study. For VWF gene, multiplex ligation dependent probe amplification assay was also done using SALSALA MLPA probein P011-B3/P012-B3. Among variants from genes of primary interest, common variant with minor allele frequency ≥1% using population databases were filtered out. In addition, variants detected in more than 2% in in-house database were further filtered out to remove population specific polymorphism or platform specific errors. For VWF exons of either incomplete coverage or low mapping quality due to highly homogeneous region (exon 26, 24), additional Sanger sequencing was performed. Genes of primary interest were those associated with platelet dysfunction (GPBA, GPB, GPI, GPIX, ADAMTS13, ITGA2B, ITGB3), Bernard-Soulier syndrome (GPAB), glanzmann thrombasthenia (ITGA2B, ITGB3), Tromboxane A2 receptor defect (TBX2A2), ADP receptor defect (P2RY12), Gaucher platelet disorder (NBEAL2), Quebec platelet disorder (PLA2U), ARC syndrome (VPS33B), Hermansky-Pudlak-G. Aleksandr2
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Background: The main clinical manifestation of primary immune thrombocytopenia (ITP) is hemorrhagic syndrome. ITP is characterized by a low platelet count or minimal cutaneous hemorrhages to severe life-threatening bleeding. It is well known, that there is no stable correlation between the platelets count or other parameter(s) and the hemorrhage grade in ITP patients. Possibly, the genetically-based individual mechanisms of immune response impairment could affect the risk of the severe ITP course. In this study, we aimed to investigate the genetic background of severe ITP.

Aims: To reveal genetic risk factor(s) for severe HS in patients with chronic ITP.

Methods: A total of 67 patients (58 women and 9 men) with chronic ITP were involved in the study. The median age of the group was 57 years (range: 24-77). The mean duration of ITP was 7 years (2-48). Hemorrhage was graded according to WHO scale. Taking into account the severity of the hemorrhage in the patients, the group was divided into two subgroups. The first group included 40 patients with HS of 0-1 grade and the second consisted of 27 patients with HS of 2-3 grade. All patients of the second group needed the use of different methods of emergency haemostatic therapy and we consider it as a “severe ITP”. We analyzed DNA polymorphism of 8 genes responsible for the formation of a human platelet alloantigen systems (HPA-1, -2, -3 and -5) or associated with impaired immune response (IL-1B, IL-6, IL-10 and TNF-A). The differences in genotype frequencies between the groups and 2 were assessed by Fisher’s exact method. Odds ratios (OR), their 95% confidence intervals (CI) and p-values were calculated with GraphPad Prism 5.0 software.

Results: The frequency of HS 3a/3a (Qpilb 2622T, 843 Ile/Ile) genotype was more than 2-fold increased in ITP patients with severe HS (55.6% vs 25.0% in the group with HS of 0-1 grade; OR=3.8, 95% Cl: 1.3-10.7, p=0.02). HPA-1a/1a and HPA-2a/2a genotypes were also more frequently seen in patients with HS 0-1 grade (23.8% vs 10.7% in HS 0-1; 92.6% vs 80.0%, respectively), but these differences were not statistically significant (p=0.78 and p=0.19, respectively). Moreover, in the group with “severe ITP” we found almost 2-fold increase of the IL-6 -174CC genotype frequency (26.9% vs 15.0% in HS 0-1; OR=2.1, 95% Cl: 0.6-7.1, p=0.34). Patients positive for IL-10 -1082A allele had a frequency of severe hemorrhages in the group with a lack of 2-3 grade (48.1% vs 26.3% in HS 0-1; OR=2.6, 95% Cl: 0.9-7.4, p=0.11).

Summary/Conclusions: Our data indicate that HPA-3a/3a variant could be a possible risk factor for severe HS in ITP patients.
P312
AN ALGORITHM TO IDENTIFY CASES OF SEVERE HEMORRHAGE IN ROUTINELY COLLECTED HEALTHCARE DATA
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Background: Many patients with a hematological malignancy have an increased risk of hemorrhages. Research addressing the causes of these hemorrhages, especially those on major hemorrhages, are hampered by the difficulty to find sufficient and representative cases of major hemorrhage. Unfortunately, electronic health records generally do not codify hemorrhages.

Aims: The aim of this study was to develop an algorithm that can be used to find patients who suffered from major hemorrhages (WHO grade 3 or 4) within electronic health records.

Methods: An algorithm was developed using electronic health record data of a cohort of patients with acute leukemia, who received platelet transfusions between June 2011 and December 2015 at the Leiden University Medical Center in the Netherlands. Chart review was performed for a stratified, random sample of observation days. Discriminative performance of three indicators was assessed: CT-brain, drop in hemoglobin level and transfusion need within 24 hours. The cut off values for hemoglobin drop and transfusion need with the best discriminating capacity and CT-brain were entered in the final algorithm. The C-statistic was calculated and calibration plots were made. The algorithm will be externally validated in two other academic hospitals.

Results: The derivation cohort consisted of 255 patients comprising 10,638 observation days and chart review was performed for 353 days. The incidence of major hemorrhage was 0.22 per 100 observation days. The final algorithm consisted of information on CT-brain (yes/no), a hemoglobin drop of >2.8 g/dl and the need of six or more transfusions (yes/no). The C-statistic of the algorithm was 0.93 (95% confidence interval (CI) 0.86 to 0.99). The incidence of bleedings with all grades of severity was 8.4 per 100 days. The algorithm for bleedings of all grades had a c-statistic of 0.54 (CI 0.53 to 0.55). The results of the external validation are not available yet.

Summary/Conclusions: An algorithm using information on CT-brain, hemoglobin drop and transfusion can accurately identify cases of major hemorrhage within electronic health care data. External validation will be performed.

P313
MOLECULAR MECHANISMS AND CLINICAL SIGNIFICANCE OF REDUCED PTPN1 EXPRESSION IN MYELODYSPLASTIC SYNDROMES
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Background: Previously we determined common deleted region (CDR) of del(20q) observed in MDS by CGH-array. Our data showed that the PTPN1 gene is located within CDR of del(20q). The PTPN1 gene encodes PTP-1B, a non-receptor type protein tyrosine phosphatase, which is involved in multiple physiological and pathological cellular processes via dephosphorylation of several tyrosine kinases, and other molecules. Although roles of PTP-1B in normal and pathological hematopoiesis has not been elucidated, it may function negative regulator for cellular processes mediated by tyrosine kinases, including Jak2, and Src. We hypothesized that the PTPN1 gene is a target gene disrupted by del(20q), resulting in haplo-insufficiency, and involved in MDS molecular pathogenesis.

Aims: We attempted to examine PTPN1 expression level in bone marrow cells of MDS patients with or without del(20q), and to investigate its clinical and biological significance.

Methods: Total RNA was extracted for cDNA synthesis from bone marrow samples taken at the time of diagnosis with written informed consent from patients and control subjects were used for the present study. Real-time RT-PCR was carried out to quantify PTPN1 expression by the TaqMan probe method using an ABI 7500 real-time PCR system (Applied Biosystems). Data including patients’ demographic, disease status, medical history, clinical and laboratory findings, and outcome, were collected from medical records and laboratory data base. A non-parametric Mann-Whitney-Wilcoxon test was used to examine whether expression levels among groups are statistically different. The Kaplan-Meier model was used to analyze the impact of PTPN1 expression on overall survival, and log-rank test was used for statistical analysis. We also examined the effect of 5-azacytidine treatment on PTPN1 expression in primary bone marrow cells from MDS patients. Bone marrow cells were cultured with or without 5mM of 5-azacytidine for 48 hours. Expression level of PTPN1 was examined by quantitative RT-PCR described as above.

Results: A total of 118 MDS patients, 71 males and 47 females with median age of 68 years (range: 20-91 years) and 19 control subjects were included in the present study. The patients were classified as RCUD (n=18), RCMD (n=58), RARS (n=8), RAEB-1 (n=20), and RAEB-2 (n=14) according to WHO classification. Relative PTPN1 expression level was significantly decreased in MDS patients with del(20q) (P<0.001) compared with control subjects. Moreover, relative PTPN1 expression level in MDS patients without del(20q) also significantly decreased (P<0.001). Expression patterns of PTPN1 among five WHO-subtypes, were statistical different (P=0.0201). Median values of relative PTPN1 expression level in RCUD, RCMD, RARS, RAEB-1, and RAEB-2 were 1.52, 1.95, 1.91, 1.46, and 1.26 respectively. Relative PTPN1 expression level in WHO-subtypes with high blast counts (RAEB-1 and RAEB-2) was significantly lower than that in WHO-subtypes with less blast counts (RCUD, RCMD, RARS) (median value: 1.41 vs 1.89, P=0.0074). To investigate prognostic implication of PTPN1 expression in MDS, we analyzed impact of PTPN1 expression on overall survival (OS). Based on PTPN1 expression level, 118 patients were divided into four groups, high (Q1), intermediate (Q2, Q3), and low (Q4) quartiles. Kaplan-Meier analysis demonstrated that the lowest quartile (Q4) showed significantly worse survival compared with remaining quartiles (Q1, Q2, Q3) (P=0.048). The estimated 5-year OS rates in Q1-3 group and Q4 group were 69% and 49.8%, respectively. We examined whether PTPN1 expression is induced by 5-azacytidine in primary bone marrow cells of 17 MDS patients. Real-time PCR analyses indicated that 5-azacytidine treatment significantly induced PTPN1 expression.

Summary/Conclusions: The present study demonstrated that PTPN1 expression is reduced in MDS patients with haplo-insufficiency due to del(20q) and methylation of promoter region of the PTPN1 gene. Low PTPN1 expression is associated with advanced disease and poorer clinical outcome, indicating that PTPN1 expression level could be a useful prognostic marker in MDS.

P314
MOLECULAR MARKERS PREDICTING RESPONSE TO AZACITIDINE TREATMENT FOR MYELODYSPLASTIC SYNDROMES
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Background: DNA hypomethylating agents (HMAs) comprise standard therapy for non-transplant-candidate high-risk myelodysplastic syndromes (MDS). However, little is known about the exact mechanism of their effects to MDS or no reliable makers predicting the response to HMAs have been developed, although a recent study reported a very high response rate of TP53-mutated AML and MDS to decitabine.

Aims: The purpose of this study is to elucidate the clonal dynamics and molecular signatures that correlate with response to azacitidine therapy for MDS, focusing on the role of TP53-mutations.

Methods: We conducted a prospective multicenter trial of azacitidine treatment for high-risk MDS patients, in which the efficacy was compared between the 5- and 7-day regimens. A total of 107 patients were enrolled between 2013 and 2016. For all cases, a bone marrow specimens collected before treatment was analyzed for mutations using targeted-capture sequencing. Mutations were also interrogated after 4 cycles of azacitidine therapy in 49 (45%) cases. An additional 42 cases were analyzed for mutations who received azacitidine therapy for MDS and whose bone marrow specimens were available both before and after therapy. RNA baits were designed for detection of both onco-mutations in 67 known driver genes in myeloid neoplasms and copy number alterations on the same platform. Response was evaluated according to the IWG-2015 criteria, taking into consideration the size of clones showing the maximum allelic burden between pre- and post-treatment specimens (ΔTFC: tumor cell fraction).

Results: On average, 2.7 mutations (range 0-9) were detected per sample before azacitidine treatment. TP53 represented the most common mutational target (60%) and was followed by ARID1A (20%) and - and off-protocol cohort, respectively, followed by ASXL1, RUNX1, TET2, and SRSF2. TP53-mutated cases had significantly lower number of driver mutations (1.7 vs 3.1/sample, p<0.001) and higher number of copy number changes (9.6 vs 2.1, p=0.001), compared with unmutated cases. Clinical response was observed in 25 cases in the on-protocol cohort, including 6 complete remission (CR) (3.6%) and 19 marrow CR (17.8%) and 7 (29%) cases (all CR) in the off-protocol cohort. Notably, CR was obtained almost exclusively in TP53-mutated cases (5/6 and 5/7 CR cases in the on- and off-protocol cohort. No other mutations were associated with CR. Median time to CR was 119 days (range: 81-721), which lasted for a median duration of 217 days (range 10-783). ΔTFC was evaluable for 62 cases who had one or more follow-up specimens and carried at least one mutation in either pre- or post-treatment with an average of -0.075 (range: -0.75-0.72). ΔTFC was significantly lower in responders than non-responders (-0.18 vs -0.0002, p=0.0068) and in TP53-mutated cases (-0.25 vs 0.0008, p=0.0011).

Summary/Conclusions: Our study revealed a significant positive association of TP53 mutations with favorable responses to azacitidine for MDS, although the response was transient and the expected response rate seems to be much lower compared to that reported for decitabine. Given that decitabine is not approved for MDS in pan-European areas (e.g. EU and Japan), our results suggest a potential role of azacitidine as a key agent to improve the notoriously dismal clinical outcomes of TP53-mutated MDS tumors. Further study should be warranted to confirm its efficacy and to develop an optimal post-remission therapy to overcome the short remission period.

P316
AZACITIDINE IMPROVES OUTCOME IN HIGH RISK MDS PATIENTS WITH CHROMOSOME 7 ABNORMALITIES: RETROSPECTIVE COMPARISON OF GESMD AND GFM REGISTRIES

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Background: A benefit of treatment with azacitidine (AZA) in higher-risk (intermediate-2 and high risk by IPSS) Myelodysplastic syndromes (HR-MDS) patients with abnormalities of chromosome 7 (Abn 7) has been suggested in relatively small studies.

Aims: Our purpose was to confirm this benefit in a larger patient series.

Methods: Retrospective study of 235 HR-MDS patients with Abn 7 treated with AZA (n=115) vs best supportive care (BSC; n=120), assessing AZA treatment as time-varying variable in multivariable analysis.

Results: Seventy-four (64%) of AZA patients had de novo MDS and 41 (36%) had therapy related (secondary MDS), compared to 70 (67%) and 8 patients (10%) in the BSC group (p=0.0001). According to WHO 2008 classification, 65% in the AZA group and 48% in the BSC groups had refractory anemia with excess of blast type 2 (RAEB-2) or secondary acute myeloid leukemia (AML with <30% of blasts) (p=0.015). The AZA and BSC groups were well balanced in terms of age, gender, cytogenetic risk category, and IPSS risk. In the AZA group, 14% of patients were RAEB-2 risk and 45% intermediate-2 risk and 61% had Complex-K, 23% non-complex-7, 14% non-complex del(7q), and only 2 patients (1.8%) had non-complex 7-. Nevertheless, regarding MDS classification and MDS subtype (de novo vs secondary) was unbalanced with more patients with RAEB-2+AML (65% vs 48%, p<0.015) and secondary MDS (36% vs 47%, p<0.015), in the AZA group compared to the BSC group. Median follow-up time from diagnosis was 47.5 months (95% CI: 24.2 – 122.9) in the AZA group and 59.8 months (95% CI: 15.5 – not reached) in the BSC group (P=ns). Median time from diagnosis to AZA treatment was 2 months (range 0 – 66.2). Ninety-two patients (80%) received AZA according to the conventional 7 days every 28 days schedule whereas 20% received 5-day cycles. The median number of AZA cycles received was 5 (range, 1-32). Response to AZA: Twelve patients were not evaluable for response according to IWG 2006 criteria because no complete data was recovered. In the 103 patients evaluable for response in the AZA group, the overall RR (ORR) was 37.9% (39/103), including 14.3% CR and 23.3% SD. Among non-responders (62.1%), 27% had SD with HI, 23.3% PD, and 11.7% (n=12) had early death (8, infection; 1, bleeding, 3, unknown cause). According to cytogenetic, the ORR was 38.1% in patients with CK, 32% in patients with non-complex-7 and 46% in patients with
with non complex del(7q) (P=ns for complex vs non complex, chi-square test). The ORR was 37.5% in "de novo" and 38.4% in secondary MDS, respectively (P=ns). Impact of AZA treatment compared to BSC on overall survival: Results of this multivariable analysis of OS at different time points are presented in Table 2. Chromeosome 7 cytogenetic categories and IPSS retained a poor prognosis over time with a constant value of low prognosis. AZA treatment had a favorable impact on OS during the first 3 years of treatment, compared to BSC, confirming results obtained in univariable analysis. Nevertheless, the benefit of AZA treatment as compared to BSC approach decreased as time spends and the HR value increased over time: HR of 0.3 at 6 months, 0.5 at 1 year and 0.7 at 2 and 3 years after treatment. (Figure 1). This benefit was present in all chromosome 7 categories with a a trend towards better impact among patients with complex karyotype but no significant differences between the 3 categories (-7, del(7q) and CK).

Figure 1.

Summary/Conclusions: This study confirms the benefit of AZA treatment on outcome in patients with HR-MDS and cytogenetic abnormalities involving chromosome 7.

P317

UN UPDATE OF A PHASE II EXPLORATORY STUDY OF OPN-305, A TOLL-LIKE RECEPTOR 2 ANTIBODY, IN PATIENTS WITH LOWER RISK MYELODYSPLASTIC SYNDROMES WITH PRIOR HYPOMETHYLATING AGENT THERAPY

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Background: Alterations of innate immune signaling, including overexpression of TLR2, are common in MDS. Significant TLR2 overexpression in MDS bone marrow CD34+ cells, especially after HMA therapy, has been reported. OPN-305 is a fully humanized antagonistic IgG4 kappa monoclonal antibody to TLR2 which significantly increases the formation of erythrocytoid colonies (CFU-E) in BM CD34+ cells isolated from pts with lower-risk MDS in vitro. Aims: To evaluate the potential therapeutic value of OPN-305 in patients (pts) with MDS.

Methods: We designed a phase I/II trial of OPN-305 for pts with low or int-1 risk MDS by IPSS after failure to prior therapy with a HMA (14 cycles). Pts whose disease was to be transduced dependent (8 units in 8 weeks). Pts with isolated del(5q) should have received therapy with lenalidomide. Because, OPN-305 had not been previously used in pts with hematological malignancies, the study had an initial phase of N=10 pts using OPN-305 at a dose of 5 mg/kg every 4 weeks for a maximum of 9 cycles. Therapy could be repeated as long as there was no excess toxicity or progression. If after 16 weeks of therapy, there was no response, azacitidine on a 3 day schedule, could be added to OPN-305. Responses were evaluated following the revised 2006 IWG criteria. This initial cohort allowed evaluation of toxicity, pharmacokinetic analysis, receptor occupancy, and sequential analysis of cytokine profile. An extension dose escalation phase to 10 mg/kg was planned for N=30 pts.

Results: At the time of this report, 31 pts have been enrolled, 11 at the initial 5 mg dose and 21 at 10 mg/kg. A total of 21 pts are evaluable for toxicity and response. Median age was 72 years (range 42-87). Eight (43%) pts were classifed as Low risk and 12 (57%) as Intermediate-1 risk by IPSS. There was no evidence of cellular redistribution (ALUG) or antibody bodies. Compared with baseline, no significant changes of IL-23, IL-18, INF-γ, IL-10, IL-18, IL-6, IL-12 (p40), IL-12 (p70) and IL-8 levels where observed among responders or non-responders or based on OPN-305 dosing. A trend to increased response was observed in patients with high TLR2 expression, 305 administration. There is no evidence of treatment related anti-drug antibody formation. The ORR including 20% transfusion independence, and potential association between TLR2 levels and response.

Summary/Conclusions: Treatment with OPN-305 in pts with previously treated lower-risk MDS was well tolerated with no significant toxicities and 53% ORR including 20% transfusion independence, and potential association between TLR2 levels and response.

P318

IN PATIENTS UNDEGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR MDS DEVELOPMENT OF CHRONIC GVHD COULD AMELIORATE THE ADVERSE IMPACT OF SPECIFIC SOMATIC MUTATIONS


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Background: Approximately 90% of patients with Myelodysplastic Syndromes (MDS) have somatic mutations in driver genes detected by Next Generation Sequencing (NGS). In the last years, several studies have related these mutations with prognosis, disease characteristics and response to therapy, including allogeneic Hematopoietic Stem Cell Transplantation (HSCT). Development of Chronic Graft Versus Host Disease (cGVHD) has been reported as one of the most powerful antineoplastic mechanisms after HSCT.

Aims: To evaluate the impact of specific somatic mutations in patients with MDS undergoing HSCT and if the development of cGVHD can modify their effect.

Methods: The results of HSCT in 115 MDS patients from five centres in Spain were retrospectively analyzed. Bone marrow samples were collected a median of 27 days prior to transplant and DNA was screened for somatic mutations by NGS, using a NextSeq platform (Illumina). Two myeloid panels that included the most frequently mutated genes in myeloid malignancies were used.

Results: Median age was 53 years (range from 19 to 70). Fifty-eight percent were male and 79.13% were classified as de novo MDS. According to WHO 2008 classification 4 (3.5%) were RCUD, 2 (1.8%) RARS, 22 (19.5%) RCD, 34 (24.8%) RAEB-1, 12 (28.3%) RAEB-2, 12 (28.3%) Unclassifiable MDS. 9 (8%) CML and 4 (3.5%) were AML (FAB RAEB-T). Among patients with calculated Revised IPSS (R-IPSS) (85 of 115 patients) 2 (2.4%) had very low risk, 15 (17.6%) low risk, 21 (24.7%) intermediate risk, 22 (25.9%) high risk and 16 (18.6%) had very high risk; 9 patients with CMLM (10.6%) were categorized into high risk group. Among patients with known karyotype (101 of 115), 20 patients had a complex karyotype (CK). Among patients with more than 2 mutated genes (47), 5% had more than 3 mutated genes (11). Patients that didn't shown any mutation before transplant; 27 patients (23.5%) had 1 mutated gene, 15 (13%) had 2, 19 (16.5%) had 3, 6 (5.2%) had 4, 3 (3.2%) had 5 and only 1 patient (0.9%) had 6 mutated different genes. The most frequently mutated genes were: TET2 in 14 patients (13%), SRSF2 in 14 (12.2%), TET2 in 13 (11.3%), SF3B1 in 9 (7.8%), RUNX1 in 9 (7.8%), FBX31 in 9 (7.8%) and ASXL1 in 8 (7%) patients. After a median of follow up for survivors of 2.02 years, Overall Survival (OS) was 48.1% (63.4% at 1 year; median 5.96). Patients were divided into 2 groups: group 1, with 2 or less mutated genes (56 patients), and group 2, with more than 2 mutated genes (59 patients). The ORR was 37.5% in 9 (7.8%) patients with a lower OS (46.9% vs 69.6% at 1 year; p=0.035) and a higher Cumulative Incidence of Relapse (CIR) (25.3% vs 10.1% at 1 year; p=0.007). Development of cGVHD significantly improved outcome in both groups (Figure 1). Univariate analysis determined that developing of cGVHD, CK, number of mutated genes (more than 2 mutated genes) and mutations in TET2 significantly impacted on outcome. Nevertheless, only the development of cGVHD as a time-dependent variable (HR 0.046, 95%CI 0.016-0.138, p=0.001) and TET2 mutations (HR 2.562, 95%CI 1.018-6.447, p=0.046) significantly influenced on OS in multi-

100 | haematologica | 2017; 102(s2)
multivariate analysis. We also observed the unfavourable impact of TP53 mutations on relapse risk: CIR was 41.7% (95% CI 22.5-77.1) at 1 year for TP53 mutated vs 9.8% (95% CI 5.3-18.1) at 1 year for non TP53 mutated patients (p=0.006).

Figure 1.

Summary/Conclusions: We conclude that the number of mutated genes prior to transplant could be a prognostic factor of OS and CIR. Mutations in some genes, like TET2 and TP53, could also have an adverse impact on outcome. However, cGVHD could ameliorate the poor prognosis of somatic mutations in transplanted patients with MDS.

P319

VOSAROXIN PLUS AZACITIDINE TREATMENT FOR PATIENTS WITH MYELODYSPLASTIC SYNDROME: A PHASE 1/COHORT EXPANSION STUDY

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Background: Although hypomethylating agents are the mainstay of treatment for myelodysplastic syndromes (MDS), these agents result in remissions in a minority of patients and are not curative. Vosaroxin is a first-in-class quinolone derivative that intercalates DNA and inhibits topoisomerase II. Vosaroxin is active with a tolerable safety profile in acute myeloid leukemia (AML) and the novel combination of vosaroxin and azacitidine was found to be synergistic in primary myeloblasts.

Aims: This phase 1/cohort expansion study was designed to determine the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of vosaroxin when given in combination with azacitidine, and to evaluate the efficacy and safety of the combination treatment.

Methods: Patients with MDS ≥18 years old with cytopenias requiring transfusions, an IPSS score of intermediate (INT)-1 or greater, or chronic myelomonocytic leukemia were eligible. Vosaroxin (initial dose: 50 mg/m²/d) was administered on Days 1 and 4, and azacitidine (75 mg/m²/d) on Days 1-7 of a 28-day cycle, in an outpatient setting, for up to 6 cycles in a 3+3 design (additional cycles were permitted if a clear benefit for the patient was demonstrated). Once the MTD was determined, an expansion cohort of 20 evaluable patients (≥1 cycle) was enrolled.

Results: A total of 35 patients enrolled in the dose escalation (n=13) and expansion (n=22) phases. The median age of the entire cohort was 66 years (range 38-77) with IPSS scores of low (n=1); INT-1 (n=13); INT-2 (n=15); and high risk (n=6). The median ECOG score for the entire cohort was 1 (range 0-2). In the dose escalation phase, at the initial dose of vosaroxin 50 mg/m²/d (n=6), the median number of total cycles was 2 (range: 1-4); 2 of 6 patients experienced a DLT at this dose (grade 4 hyperbilirubinemia and grade 4 neutropenia >42 days). At the de-escalated dose of 34 mg/m²/d (n=7), the median number of cycles was 2 (range: 1-18); 1 patient experienced a DLT at this dose (grade 4 mucositis). The MTD of vosaroxin was determined to be 34 mg/m²/d when given on Days 1 and 4 with a fixed dose of 75 mg/m² of azacitidine on Days 1-7. The major non-hematologic toxicities were infections, febrile neutropenia, and bleeding. The combination of vosaroxin and azacitidine showed promising activity with responses rates comparable or better than those generally observed with azacitidine alone. Additionally, the transplant rate observed was encouraging in this patient population.

Table 1.

Summary/Conclusions: The MTD of vosaroxin in MDS patients was 34 mg/m²/d when given on Days 1 and 4 with a fixed dose of 75 mg/m² of azacitidine on Days 1-7. The major non-hematologic toxicities were infections, febrile neutropenia, and bleeding. The combination of vosaroxin and azacitidine showed promising activity with responses rates comparable or better than those generally observed with azacitidine alone. Additionally, the transplant rate observed was encouraging in this patient population.
Myeloma and other monoclonal gammopathies - Biology

P320

ADVANCED STAGE MYELOMA IS CHARACTERIZED BY A SIGNIFICANT INCREASE OF MUTATIONS IN GENES ASSOCIATED WITH DRUG RESPONSE

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Background: The amount of genomic data available in Multiple Myeloma (MM) is exponentially increasing, however, hardly any of that information is translated into the clinic. A number of genes has been associated with resistance to commonly used anti MM compounds. This, most importantly, includes immunomodulators (IMiDs) and proteasome inhibitors (PIs). However, no mutation screening has yet been amended to our MM routine diagnostic workflows. We investigated 458 MM patients by targeted sequencing, including the largest cohort of previously treated MM patients so far. We identified an increased mutation incidence in treated patients, yet unreported mutations and functionally validated a subset.

Aims: To describe the mutational spectrum in genes of pathways targeted by standard of care (SOC) therapies in a cohort of pretreated and previously untreated patients.

Methods: Tumor-germline paired samples of five contributing sites were pooled (Würzburg, Heidelberg, Madrid, Rotterdam and Mayo Clinic). Analysis included 310 untreated and 148 IMID and/or PI treated patients. Targeted sequencing was performed using the M3P (v2.0 or v3.0) gene selection, that includes most commonly mutated MM genes, actionable drug targets and genes being associated with drug resistance. Average sequencing depth increased 700X. Functional analyses of PSMB5 mutations were conducted using Sleeping beauty vectors transposed into AMO1 cell line.

Results: Our analysis included five genes each with known association to drug response to IMiDs (CRBN, CUL4B, IKZF1, IKZF3 and IRF4) and PIs (PSMB5, PSMB8, PSMB9, PSMD1 and XBP1). Based on the increased sequencing depth, the mutation incidence in untreated patients is higher than in the CoMMPass database (IMiDs: 5.8% vs 3.9%; PIs: 1.9% vs 1.4%). Furthermore, pretreated patients showed a significant mutational increase compared with untreated pts (IMiDs: 19.7%, Z-score: -4.2, p<0.001; PIs: 7.3%, Z-score: -2.6, p=0.009). We observed a Gly159Arg mutation within the Lealendim (Len) degron sequence of IKZF3 in a patient progressing on Len and Pomalidomide (Pom), as well as two XBP1 truncating mutations in PI refractory patients. Of note, among three treated cases with mutations in the β5 (PSMB5) or βi (PSMB8) PI binding subunit of the proteasome, one patient harbored not less than 4 subclonal mutations. This is the first description of PSMB5 mutations in human MM, identified in a patient with long term history of PI treatment. All mutations were located in or close to the Bor binding site of PSMB5. The functional analysis demonstrated induction of resistance not only to Bor (IC50wt=2 nM vs IC50mut=4.5-8 nM), but also to the second generation PI Ixazomib (IC50wt=5.2 nM vs IC50mut=N/A) and Carfilzomib (IC50wt=8 nM vs IC50mut=13-22 nM). Of interest, the P97 blockade of the protein homeostasis by the investigational compound CB5083 remains still possible in the mutated cell lines and the resistance can be overcome. Finally, Pom treatment eradicated two of the PSMB5 containing subclones (Figure 1).

Summary/Conclusions: Under the selective pressure of anti-MM therapy the incidence of mutations in genes associated with drug resistance increases in resistance mechanisms, evolve in parallel in competing (sub)clones of the disease, mimicking phenotype and behavior. Remarkably, despite our restrictive gene selection, a quarter of our treated cohort is affected by at least one mutation. Aim of future therapy may be the eradication of selected clones or subclones, which, according to our data, appears possible.

P321

ILF2-YB1 INTERACTION MODULATES RNA SPlicing TO INDUCE RESISTANCE TO DNA-DEAMAGING AGENTS IN 1Q21-AMPLIFIED MULTIPLE MYELOMA


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Background: The 1q21 amplification, which occurs in approximately 40% of de novo and 70% of relapsed Multiple Myeloma (MM), is among the most frequent chromosomal aberrations in MM patients and is considered a very high risk genetic feature that is especially correlated with disease progression and drug resistance. The 1q21 amplicon contains many genes, and while it is unlikely that all contribute to the pathobiology of high-risk MM, the critical genes that do drive this high-risk phenotype have not yet been fully clarified. Identifying such genes and their contributions to this phenotype would enable the development of new and effective targeted therapy strategies for high-risk MM and thus improve their survival outcomes.

Aims: In our study we wanted to investigate the biological and molecular mechanisms behind the 1q21 amplification’s contribution to high-risk MM with the ultimate goal of obtaining a list of validated therapeutic targets to inform the design of novel translational clinical trials for this subgroup of patients.

Methods: We conducted a high-resolution analysis of recurrent copy number alterations and expression profiles in a collection of 254 MM samples included in MMRC database. To define the discrete minimal common 1q21 region that is recurrently amplified in MM, we used Genomic Identification of Significant Targets in Cancer, a systematic method that identifies regions of genome that are recurrently amplified or deleted across a set of samples. These regions were enlisted into an in vitro screening strategy that employed a single-stranded-96-well approach and GFP-competitive cell growth assay to identify 1q21 genes whose loss of function resulted in the selective death and/or growth inhibition of MM cells carrying the 1q21 amplification but not MM cells without the 1q21 amplification.

Results: We identified MCL1, UBAP2L, INSTM3, LASS2, KRTCAP2 and ILF2 as potential 1q21-specific vulnerability targets whose expression is driven by copy number. We functionally validated, both in vitro and in vivo, Interleukin-2- enhancer binding factor 2 (ILF2) as a key 1q21 amplification-specific gene. Our results show that ILF2 interacts homologously with the HRD complex (BRCA1 and RNF4) and induces resistance to DNA damaging agents routinely used in the treatment of MM, which is consistent with the observation that ILF2 expression correlates with poor survival in MM patient treated with high-dose melphalan followed by tandem autologous transplantation. On the mechanistic level, ILF2 interacts with numerous RNA binding proteins directly involved in the regulation of DNA Damage Response (DDR) by modulating alternative splicing of specific pre-mRNAs. RNA sequencing experiment confirmed that ILF2 knockdown results in aberrant splicing of genes involved in the DDR pathways and, strikingly, ILF2 RIP-seq analysis showed that ILF2 directly binds to transcripts involved in the regulation of the HR pathway, including components of BRCA1 protein complex. Furthermore, we found that ILF2 mediates drug resistance in dose-dependent manner by modulating YB-1 nuclear localization and interaction with the splicing factor U2AF65 to promote mRNA processing and stabilization of DDR genes in response to DNA damage (Figure 1).

Summary/Conclusions: In conclusion, our study reveals an intimate relationship among 1q21 amplification, mRNA splicing and DNA repair in the control of DDR in MM. On the basis of our findings, we propose that 1q21-driven ILF2 overexpression deregulates HR by stabilizing the mRNA splicing of critical HR
effectors, which enables genomic instability, promotes adaptive mechanisms to genotoxic stress, and enhances cell survival, thereby promoting drug resistance and disease progression. Given that 1q21 amplification is one of the most frequent copy number alterations in cancer, synthetic lethality approaches based on targeting gain-of-function associated to ILF2 may have a broad spectrum of application to potentiate the sensitivity of cancer cells to chemotherapeutic agents.

Figure 1.

P322

PROGNOSTIC IMPLICATION OF SOMATIC MUTATIONS BY NEXT GENERATION SEQUENCING: AN ANALYSIS FROM THE MMRF COMMPASS STUDY IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

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Background: High throughput techniques, such as next generation sequencing, are becoming an appealing approach to characterize multiple myeloma (MM) genomic profiles and better define risk assessment. However, the clinical relevance of such approaches is still largely unknown. The Multiple Myeloma Research Foundation (MMRF) CoMMpass trial (NCT01454297) has collected data from 1154 newly-diagnosed MM patients enrolled worldwide. Comprehensive analysis of somatic mutations in MM cells at diagnosis could unravel prognostically relevant disease characteristics not detectable with traditional approaches.

Aims: We analyzed data from the interim analysis 8 cohort (August 2015) to create a prognostic model.

Methods: CD138+ purified MM specimens from bone marrow aspirates and peripheral blood cells were collected at diagnosis. Whole exome libraries from both tumor and constitutional DNA samples were created. Somatic single nucleotide variants (SNV) were identified, only nonsynonymous SNV were included in the analysis. We evaluated the impact on progression free survival (PFS) of recurrently mutated genes (with at least a nonsynonymous SNV with an allelic frequency of more than 5% in more than 10 patients) in a multivariable Cox model (NCT01454297) that has collected data from 1154 newly-diagnosed MM patients enrolled worldwide. Comprehensive analysis of somatic mutations in MM cells at diagnosis could unravel prognostically relevant disease characteristics not detectable with traditional approaches.

Results: We analyzed data from the interim analysis 8 cohort (August 2015) to create a prognostic model.

Methods: CD138+ purified MM specimens from bone marrow aspirates and peripheral blood cells were collected at diagnosis. Whole exome libraries from both tumor and constitutional DNA samples were created. Somatic single nucleotide variants (SNV) were identified, only nonsynonymous SNV were included in the analysis. We evaluated the impact on progression free survival (PFS) of recurrently mutated genes (with at least a nonsynonymous SNV with an allelic frequency of more than 5% in more than 10 patients) in a multivariable Cox model adjusted for international staging system (ISS) and cytogenetic profile (high risk, standard risk and missing). A backward selection based on the Akaike Information Criterion (AIC) was used to identify the final Cox model used to create a scoring system.

Results: 517 patients with baseline somatic mutation data were included in the analysis. Median age at diagnosis was 64 years (range 27-93), all patients received novel agents as first line treatment, 236 (45.6%) received autologous stem cell transplantation (ASCT). The most recurrent mutated genes were KRAS (25%) and NRAS (19.5%). Consistently with other works, DNA allele frequency data revealed that, in the great majority of cases, only a subclonal portion of MM cell DNA harbors a selected somatic mutational status of 9 recurrently mutated genes could improve risk assessment of newly-diagnosed MM patients. Longer follow-up and validation in independent cohorts of patients are needed to confirm our findings. Updated results with a longer follow-up will be presented at the meeting.

Table 1.

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</table>

Figure 2.

Summary/Conclusions: The use of a prognostic model based on the mutational status of 9 recurrently mutated genes could improve risk assessment of newly-diagnosed MM patients. Longer follow-up and validation in independent cohorts of patients are needed to confirm our findings. Updated results with a longer follow-up will be presented at the meeting.

P323

TARGETING GENE DEPENDENCY OF 1Q AMPLIFICATION IN MULTIPLE MYELOMA

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Background: Gain of 1q is one of the most frequent copy number variations across cancer types and in Multiple Myeloma (MM). Gain of 1q is associated with a poor outcome, indicating it is a potential driver in MM progression and resistance to treatment. While the whole 1q arm can be amplified in some cases, a specific minimal amplified region has been identified by CGH array, including approximately 500 genes in the 1q21.1-23.3 region. However, the driver genes in the 1q region are unknown.

Aims: We hypothesize that specific genes present in the 1q minimal amplified region are critical regulators of clonal evolution and tumor progression in MM.

Methods: To explore gene dependency in 1q21.1-23.3 in MM, lung, breast and
ovoian cancers, we performed a shRNA targeted screen, using the CR91 technology. We used 14 cell lines, including MM, lung, and breast cancer cell lines. We designed a pooled library targeted shRNA/cR91 screen containing 6 shRNAs along with their matched control for each of the 500 genes in the 1q21.1-23.3 region, including IncRNA and miRNA in addition to protein coding genes. The pooled library contained 6500 shRNAs, including CR91 controls as a control. We validated the expression-profiling resource developed by the Library of Integrated Network-based Cellular Signatures (LINCS) program to identify potentially active drug targeting our candidate genes. Finally, a targeted drug screening was performed using 179 compounds identified through the LINCS program and using the Cell-in-a-Well technology. Compounds exhibiting significant toxicity on each of the PLAT arrays were then validated in 1q+ (OPM2, H929 and KMS11) and non 1q cell lines (KMS18).

Results: We were able to identify 10 candidate genes, for which knockdown significantly impaired proliferation of gain of 1q cell lines. Several candidate genes were identified as being the top genes preferentially affecting the proliferation of gain of 1q cell lines. To further confirm that our candidate genes are overexpressed in gain of 1q patients, we studied publicly available gene expression profiling from the Multiple Myeloma Genomic portal (MMPG) and the Cancer Genome Atlas (TCGA). Using the list of candidate genes, we identified a large expression-profiling resource developed by the Library of Integrated Network-based Cellular Signatures (LINCS) program to identify potentially active drugs targeting our candidate genes. Finally, a targeted drug screening was performed using 179 compounds identified through the LINCS program and using the Cell-in-a-Well technology. Compounds exhibiting significant toxicity on each of the PLAT arrays were then validated in 1q+ (OPM2, H929 and KMS11) and non 1q cell lines (KMS18).

Summary/Conclusions: In conclusion, our study is the first one to ever show that acquired IMiD-resistance is mainly an epigenetic event that is potentially reversible through a combination of two epigenetic compounds, 5-Azac and EPZ-6438. These drugs have been shown to have low levels of toxicity, thus making them very good candidates for a prospective phase I study to examine their potential as “IMiD-resensitizers”, which may improve the outcome treatment of MM patients with drug-resistant myeloma clones and a potentially high-risk disease.

P324
DUAL INHIBITION OF DNMT1 AND EZH2 CAN EFFECTIVELY OVERTHE BOTH INTRINSIC AND ACQUIRED RESISTANCE OF MYELOMA CELLS TO IMiDS
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Background: The introduction of novel agents for the treatment of multiple myeloma (MM), mainly proteasome inhibitors and immunomodulatory agents (IMiDs), has significantly improved the survival rates of the patients, and both classes of drugs stand as the main treatment options for MM. Several studies have identified Cereblon (CRBN) as the direct target of not only thalidomide, but also lenalidomide and pomalidomide, and suggested that its expression is essential for the anti-myeloma effect of these drugs. However, even though the expression levels of CRBN have been associated with response to IMiDs, not all studies confirm this finding, as for example patients or cell lines with high levels of CRBN might not exhibit sensitivity to IMiDs. Thus, the expression of CRBN does not consistently explain the lack of IMiD sensitivity and the precise mechanisms behind IMiD resistance still remain elusive.

Aims: The aim of this study was to examine the importance of epigenetic modifications in the expression of CRBN and other key drug resistance markers in MM cell lines. We also re-evaluated the impact of IMiD resistance, as well as investigate whether restoration of CRBN expression is feasible through epigenetic reprogramming by epigenetic modulators.

Methods: For the development of IMiD-resistant cell lines (OPM2-LR and -PR, H929-LR and -PR), we treated OPM2 and NCI-H929 continuously with increasing doses of IMiD for 4-6 months, until growth and proliferation were not affected. Nucleosome positioning (chromatin accessibility) was assessed using AccesSsible, with all the analyses performed in the statistical software R, using the package minfi. RNA-seq is currently being performed for OPM2, NCI-H929 their IMiD-resistant as well as resensitized counterparts (N=10) in collaboration with BGI, using BGISEQ500 platform. We also performed for OPM2, NCI-H929 their IMiD-resistant as well as resensitized counterparts (N=10) in collaboration with BGI, using BGISEQ500 platform.

Results: We were able to identify 10 candidate genes, for which knockdown significantly impaired proliferation of gain of 1q cell lines. Several candidate genes were identified as being the top genes preferentially affecting the proliferation of gain of 1q cell lines. To further confirm that our candidate genes are overexpressed in gain of 1q patients, we studied publicly available gene expression profiling from the Multiple Myeloma Genomic portal (MMPG) and the Cancer Genome Atlas (TCGA). Using the list of candidate genes, we identified a large expression-profiling resource developed by the Library of Integrated Network-based Cellular Signatures (LINCS) program to identify potentially active drugs targeting our candidate genes. Finally, a targeted drug screening was performed using 179 compounds identified through the LINCS program and using the Cell-in-a-Well technology. Compounds exhibiting significant toxicity on each of the PLAT arrays were then validated in 1q+ (OPM2, H929 and KMS11) and non 1q cell lines (KMS18).

Summary/Conclusions: In conclusion, our study is the first one to ever show that acquired IMiD-resistance is mainly an epigenetic event that is potentially reversible through a combination of two epigenetic compounds, 5-Azac and EPZ-6438. These drugs have been shown to have low levels of toxicity, thus making them very good candidates for a prospective phase I study to examine their potential as “IMiD-resensitizers”, which may improve the outcome treatment of MM patients with drug-resistant myeloma clones and a potentially high-risk disease.

P325
MULTILAYER EPIGENOMIC ANALYSES REVEAL OF NEW CANCER ONCOGENES INVOLVED IN THE PATHOGENESIS OF MULTIPLE MYELOMA
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Background: Most of the published omics studies in multiple myeloma (MM) have focused on the analysis of the genome, transcriptome and DNA methy- lome. Over the last years, the chromatin structure and histone modifications are emerging as essential epigenetic layers to understand gene deregulation in MM, although this field remains widely unexplored in MM.

Aims: We herein aim to elaborate a comprehensive description of the MM epigenome including multiple layers of information.

Methods: We performed ChiP-seq of six histone modifications with non-over- lapsing functions (H3K4me3, H3K4me1, H3K27ac, H3K36me3, H3K27me3, and H3K9me3), ATAC-seq for chromatin accessibility, Whole Genome Bisulfite Sequencing (WGBS) for DNA methylation, and RNA-seq for gene transcription in purified bone marrow plasma cells from four MM patients and, as healthy controls, naive B cells, germinal center B cells, memory B cells and plasma cells. Data were extensively mined using a battery of different bioinformatic tools.

Results: We were able to identify 10 candidate genes, for which knockdown significantly impaired proliferation of gain of 1q cell lines. Several candidate genes were identified as being the top genes preferentially affecting the proliferation of gain of 1q cell lines. To further confirm that our candidate genes are overexpressed in gain of 1q patients, we studied publicly available gene expression profiling from the Multiple Myeloma Genomic portal (MMPG) and the Cancer Genome Atlas (TCGA). Using the list of candidate genes, we identified a large expression-profiling resource developed by the Library of Integrated Network-based Cellular Signatures (LINCS) program to identify potentially active drugs targeting our candidate genes. Finally, a targeted drug screening was performed using 179 compounds identified through the LINCS program and using the Cell-in-a-Well technology. Compounds exhibiting significant toxicity on each of the PLAT arrays were then validated in 1q+ (OPM2, H929 and KMS11) and non 1q cell lines (KMS18).

Summary/Conclusions: In conclusion, our study is the first one to ever show that acquired IMiD-resistance is mainly an epigenetic event that is potentially reversible through a combination of two epigenetic compounds, 5-Azac and EPZ-6438. These drugs have been shown to have low levels of toxicity, thus making them very good candidates for a prospective phase I study to examine their potential as “IMiD-resensitizers”, which may improve the outcome treatment of MM patients with drug-resistant myeloma clones and a potentially high-risk disease.
cells. Out of this list, we observed that two adjacent genes, PRDM5 and NID1, were co-activated in MM. The analysis of their expression in additional patient cohorts indicated that their co-regulation is a consistent event in MM pathology and that their levels were negligible in bone marrow and tonsillar plasma cells. When analyzing chromatin topology by 4C-Seq, we identified 3D interactions between both gene loci only in MM cells, suggesting that DNA looping between the two genes may be related to their co-activation in MM. Finally, knockdown of each of these genes using inducible shRNAs, decreased cell proliferation and induced apoptosis in MM cells.

Summary/Conclusions: Collectively, our initial exploration of histone modification profiles in MM has revealed an extensive activation of the MM chromatin landscape, which harbors a few candidate oncogenes. Reversing this global activation by epigenetic drugs, such as BET inhibitors, may represent an attractive therapeutic option for MM.

P326

CLINICAL IMPLICATIONS OF CLONAL CD34+ CELLS IN STEM CELL HARVEST FROM PATIENTS WITH PLASMA CELL DYSCRASIAS

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Background: Introduction of novel treatments; Lenalidomide, high-dose alkylating agents (Melphalan) conditioning prior to autologous stem cell transplant (ASCT) over the last few decades has improved overall survival in patients with multiple Myeloma (MM). In spite of enhanced survival rates, some hematological malignancies (SPM) like Myelodysplastic syndrome (MDS) and Acute myeloid Leukemia (AML). Clonal haematopoiesis resulting in sequential accumulation of a combination of driver-passenger genetic mutations (in up to 80% of MDS & <95% AML patients) steer MDS/AML disease pathogenesis and clinical outcome. Therefore, we hypothesised that detection of Clonal Haematopoiesis of Indeterminate Potential (CHIP) in haematopoietic stem cells (HSCs) prior to ASCT in patients with MM treated with a range of therapies could be utilised for predicting patients at risk of developing SPMs i.e. MDS/AML.

Aims: To ascertain baseline mutation spectrum [especially low-level clones with variant allele frequency (VAF) ≥5%] of MDS/AML associated gene mutations in HSCs prior to ASCT in order to predict patients at risk of clonal evolution, transformation to MDS/AML.

Methods: DNA was isolated from mononuclear cells (MNCs) collected by leucopheresis prior to ASCT from 128 MM patients. A customised amplon-based Illumina MiSeq panel was used for the sensitive interrogation of 24 most common genes harboring mutations in MDS/AML (splicing factor genes; SF3B1, SRSF2, U2AF1 and ZRSR2, genes implicated in epigenetic regulation; TET2, IDH1, IDH2, TET2 & DNMT3A, known cancerogenesis genes involved in cell signalling/transcription regulation and cohesion complex; TP53, FLT3, NRAS, KRAS, ETVD, RUNX1, CCBL, C-KIT, JAK2, MPL, CEBPA, STAG2, GATA2, KDM6A and NPM1). Variant analysis was performed using Illumina Variant Studio (≥5% VAF & read depth ≥150X threshold). To accommodate for the lack of germ line material to confirm the somatic nature of the variants, SNPs occurring at a frequency of ≥0.001% in the threshold). To accommodate for the lack of germ line material to confirm the somatic nature of the variants, SNPs occurring at a frequency of ≥0.001% in the population [e.g. dbSNP132, UCSC genome browser, Exome sequencing project (esp6500), Exome Aggregation Consortium (Exac)] were excluded.

Results: Seven patients (5.625%) contained heterozygous somatic mutations (VAF range 7-50%) in DNMT3A, IDH1, IDH2, TET2, ETVD and CBL genes (Table 1). Four missense mutations identified in DNMT3A were aggregated in the Mtese domain responsible for its methyltransferase activity indicating a strong intent to abrogate this function. Previous studies confirm R882 variant (accounts for ~60% DNMT3A mutations) as a founder lesion in MDS/AML stratum associated with clonal haematopoietic disease. HSC differentiation missense mutation in CBL (I429F) has been previously reported in CML cases (while translocations and deletions of ETVD are more common in AML M0 (5%) compared to mutations suggesting it’s role as a tumor-suppressor . Genes identified in our cohort are frequently associated with MDS & AML; IDH1/IDH2 (5 & 20%), TET2 (12 & 20%), DNMT3A (8 & 20%) and associated with poor prognosis (DNMT3A, IDH1/IDH2). SNP array karyotyping on 4/7 cases (patients 1-4) displayed no chromosomal abnormalities. Median age at diagnosis in these four cases was 65 (range 60-70), long-term follow up (3-5 yrs) revealed relapse of MM in patient 1 and 3, acute kidney injury with myeloma in patient 2 and transformation to AML in patient 4.

Summary/Conclusions: Our data identifies for the first time a subgroup of MM patients (6.25%) with no morphological evidence of MDS/AML prior to ASCT but harbouring CHIP in CD34+ harvest stem cells and later developing MDS/AML. These findings are pivotal for identification of such patients at risk of clonal evolution and transformation prior to ASCT since it can be a significant parameter in determining appropriate treatment modality i.e. whether or not to employ CHIP harbouring CD34+ harvest stem cells as therapy for these patients.

P327

PATHOPHYSIOLOGICAL FUNCTIONS AND CLINICAL IMPACT OF THE NEW IMMUNORECEPTOR SLAMF3 IN MULTIPLE MYELOMA

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Introduction of novel treatments; Lenalidomide, high-dose alkylating agents (Melphalan) conditioning prior to autologous stem cell transplant (ASCT) over the last few decades has improved overall survival in patients with multiple Myeloma (MM). In spite of enhanced survival rates, some hematological malignancies (SPM) like Myelodysplastic syndrome (MDS) and Acute myeloid Leukemia (AML). Clonal haematopoiesis resulting in sequential accumulation of a combination of driver-passenger genetic mutations (in up to 80% of MDS & <95% AML patients) steer MDS/AML disease pathogenesis and clinical outcome. Therefore, we hypothesised that detection of Clonal Haematopoiesis of Indeterminate Potential (CHIP) in haematopoietic stem cells (HSCs) prior to ASCT in patients with MM treated with a range of therapies could be utilised for predicting patients at risk of developing SPMs i.e. MDS/AML.

Aims: To clarify this, we investigated the expression and functions of SLAMF3 in MM.

Materials: 1) Two hundred thirty patients comprising 153 newly diagnosed (19 asymptomatic and 134 symptomatic) MM patients, 30 refractory/relapsed MM patients, and 47 patients with monoclonal gammopathy of undetermined significance were enrolled. SLAMF3 and CD138 expression levels on clonal plasma cells were analyzed using flow cytometry (FCM). Soluble SLAMF3 (sSLAMF3) plasma levels were measured using ELISA. 2) Drug sensitivity to anti-myeloma agents (melphalan and bortezomib) and the proliferation potential in MM cell lines KMS18 and U266 were analyzed using FCM and the MTT assay. SLAMF3 knockdown MM cell lines were obtained using the lentiviral shRNA system and siRNA. Stable transfected KMS34 cell lines overexpressing full-length SLAMF3 and cytoplasmic-domain-truncated SLAMF3 (ΔSLAMF3) were established through corresponding vectors. Single-nucleotide polymorphism (SNP) genotyping was analyzed by real-time PCR. The adaptor protein of SLAMF3 was identified by Western blotting and immunoprecipitation.

Results: 1) SLAMF3 was highly expressed on plasma cells in almost all MM patients, even in relapsed/refractory disease, although CD138 expression levels were decreased in some with advanced disease. 2) The proliferative potential and percentage of antitymoma agent-induced apoptosis in SLAMF3+ MM patients were significantly higher and lower than in SLAMF3− MM cells, respectively. The cell proliferation and drug resistance in SLAMF3− MM patients were higher than those in SLAMF3+ MM cells. 3) The frequency of GG genotypes of SLAMF3 SNP rs509749 in MM patients was 63.6% (n=28), of AG 29.5% (n=13), and of AA 6.8% (n=3). Patients with AG genotypes tended to have shorter overall survival times than patients with GG genotypes. 4) sSLAMF3 levels were significantly higher in symptomatic MM than in asymptomatic MM and markedly increased in advanced MM. MM patients with high levels (≥3.3 ng/mL) of sSLAMF3 progressed to the
advanced stage significantly more often and had shorter progression-free survival times than those with low levels (p=0.032).

Summary/Conclusions: This study revealed that SLAMF3 molecules consistently expressed on MM cells may transmit positive signals mediated via the complex of SHP2 and GRB2 by self-ligand interaction between MM cells and induce a high malignant potential in MM. Furthermore, high levels of serum sSLAMF3 may reflect MM disease progression and be a useful prognostic factor in MM. Thus, SLAMF3 molecules may be a new potential target for future immunotherapy and chemotherapy.

P328

TARGETING CD74 IN MULTIPLE MYELOMA WITH A NOVEL ANTIBODY DRUG CONJUGATE, STRO-001

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Background: CD74 is a transmembrane glycoprotein involved in MHC protein formation and transport. CD74 expression has been observed in up to 90% of B-cell malignancies, including multiple myeloma (MM), with minimal expression in normal tissues. CD74 is rapidly internalized, making it an attractive target for ADCs. STRO-001 is a novel ADC comprised of an aglycosylated anti-CD74 IgG1 human antibody (SP7219) conjugated covalently to the non-natural amino acid para-azido-methyl-L-phenylalanine (pAMF) with a non-cleavable dicyclooctyne (DBCO)-maytansinoid linker-warhead. Highly efficient site-specific conjugation enabled by novel cell-free antibody production and click chemistry results in in vitro cytotoxicity and in vivo efficacy of STRO-001 in MM cell lines and reduces tumor burden in MM xenograft models, including significant prolongation of survival in the MM.1S model. Based on these encouraging observations, STRO-001 is advancing to the clinic for the treatment of CD74-expressing B-cell malignancies.

Summary/Conclusions: STRO-001 demonstrates potent in vitro cytotoxicity in MM cell lines and reduces tumor burden in MM xenograft models, including high malignant potential in MM. Furthermore, high levels of serum sSLAMF3 may reflect MM disease progression and be a useful prognostic factor in MM. Thus, SLAMF3 molecules may be a new potential target for future immunotherapy and chemotherapy.

P329

GENOTYPE CHARACTERIZATION OF LIGHT CHAIN AMYLOIDOSIS BY WHOLE EXOME SEQUENCING

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Background: Immunoglobulin light-chain amyloidosis (AL) is a heterogeneous and multifactorial disease with high genetic complexity. Until now, no common factor or unique mutation associated with this disease has been described. Whole exome sequencing in Multiple Myeloma (MM) patient’s tests allowed to know important genes and pathways that are involved in the disease. However, few evidences through next generation sequencing (NGS) analysis were described in AL. Consequently, the application of NGS technologies permits unraveling the genomic landscape of AL to better disentangle the biology of the disease, allowing the identification of new therapeutic targets as in MM.

Aims: Genotype characterization of novel molecular alterations in AL plasma cell by whole-exome sequencing technology.

Methods: We studied 40 paired samples (sorted pathological plasma cells and peripheral blood) from 20 patients with AL. Whole exome and regulatory regions were captured using Agilent’s SureSelect Human All Exon V6+UTR kit and sequenced on the Illumina NextSeq 500 platform with pair-end sequencing technique with a global mean depth coverage of 70x. Data were analyzed with wANNOVAR for functional annotation, and a data reduction strategy to identify candidate variants. Differences that were observed in expression between patients and controls were analyzed with differential expression analysis (DESeq2). For copy number variation, we used the Strelka software to discard germinal mutations.

Results: After the analysis of patient samples we got an average of 76 (range 18-177) mutations per patient. 28.4% of the mutations was located on regulatory regions. The mutation pattern was very heterogeneous between patients. We identified differences in genes involved in extracellular matrix (MMP2), cell proliferation, differentiation and development (TFGA), transcription factors (ZFHX3, HNRP-A), and peripheral blood) from 20 patients with AL. Whole exome and regulatory regions were captured using Agilent’s SureSelect Human All Exon V6+UTR kit and sequenced on the Illumina NextSeq 500 platform with pair-end sequencing technique with a global mean depth coverage of 70x. Data were analyzed with wANNOVAR for functional annotation, and a data reduction strategy to identify candidate variants. Differences that were observed in expression between patients and controls were analyzed with differential expression analysis (DESeq2). For copy number variation, we used the Strelka software to discard germinal mutations.

Summary/Conclusions: Taken together, these results suggest that the mutation pattern in AL is heterogeneous with no common mutated gene among all patients. However, we described novel mutations in the context of AL in regulatory genes or over-representing cancer-related pathways that can help to elucidate the molecular biology of the disease.

Summary/Conclusions: STRO-001 demonstrates potent in vitro cytotoxicity in MM cell lines and reduces tumor burden in MM xenograft models, including significant prolongation of survival in the MM.1S model. Based on these encouraging observations, STRO-001 is advancing to the clinic for the treatment of CD74-expressing B-cell malignancies.

References:
**Myeloma and other monoclonal gammapathies - Clinical 1**

**P330**

**IMPROVED SURVIVAL IN 21,465 MULTIPLE MYELOMA PATIENTS: RESULTS FROM A POPULATION-BASED STUDY**

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**Background:** Multiple myeloma (MM) is generally considered an incurable disease, however advances in the treatment options for MM have been great in recent years. Recent studies on these new agents indicate an improvement in survival, nevertheless population-based studies have had contradicting findings, especially in the elderly patients.

**Aims:** The aim of the study was to evaluate the survival of all patients diagnosed with MM in Sweden in the years 1973 to 2013 and to relate the survival pattern to trends in treatment strategies.

**Methods:** Patients diagnosed with MM in the period from January 1, 1973 to December 31, 2013 were identified from the Swedish Cancer Registry. Information on sex, date of birth, date of diagnosis, and date of death was collected. Relative survival ratios (RSRs) were used to provide a measure of excess mortality of MM patients compared to a comparable group from the general population. RSRs with 95% confidence intervals (CIs) were found for 1-, 5-, and 10-years for 4 calendar periods; 1973-1982, 1983-1992, 1993-2002, and 2003-2013 and furthermore for 6 age categories at diagnosis (0-40, 41-50, 51-60, 61-70, 71-80 and >80). Short-term survival, as defined by RSR of less than 3 months, was also defined for all calendar periods.

**Results:** A total of 21,465 patients (54% males, median age at diagnosis 72 years) with MM were recorded in the time period. Overall, the 1- and 5- and 10-year RSRs improved in the whole period, with the greatest improvement in the two most recent calendar periods. The 1-year RSR increased significantly between all calendar periods (0.69, 0.74, 0.77 and 0.82, respectively). The 5-year RSR increased significantly between the two last calendar periods (0.31, 0.33 and 0.41, respectively; Figure 1) as well as the 10-year RSR (0.10, 0.12, 0.14 and 0.20, respectively). Short-term survival increased significantly between the first two and last two calendar periods (the RSR were 0.83, 0.88, 0.89 and 0.93 respectively). Females had a lower excess mortality compared to males (excess mortality ratio 0.91).

**Summary/Conclusions:** In this population-based study, based on more than 21,000 MM patients diagnosed during more than 40-year period, we showed that with an increased use of novel agents in MM patients, survival has improved significantly. This is especially prominent during the last 10 years. Our findings are important, since new agents are approved based on clinical trials, where certain groups, such as older patients and patients with significant comorbidities are often excluded.

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**P331**

PROGNOSTIC IMPLICATIONS OF MULTIPLE CYTOGENETIC HIGH-RISK ABNORMALITIES IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA


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**Background:** Cytogenetic evaluation using fluorescence in situ hybridization (FISH) at the time of diagnosis is essential for initial risk stratification in multiple myeloma. The presence of specific cytogenetic high-risk abnormalities (HRA) is known to confer a poor prognosis, less is known about the cumulative effect of multiple such abnormalities.

**Aims:** To evaluate the prognostic implications of the presence of multiple HRA at the time of diagnosis.

**Methods:** We studied 1181 patients who were diagnosed with multiple myeloma between July 2005 and July 2015 at Mayo Clinic Rochester, underwent FISH evaluation within 6 months of diagnosis, and received first-line therapy with at least 1 novel agent (immunomodulator or proteasome inhibitor). HRA were defined as t(4;14), t(14;16), t(14;20), del(17p), and gain(1q). Bone marrow aspirates were evaluated for deletions, monosomies, trisomies, and tetrasonies using chromosome or centromere-specific FISH probes. IGH rearrangements were evaluated using an IGH break-apart probe and evaluating up to 5 potential partners (FGFR3, CCND1, CCND3, MAF, and MAFB). Kaplan-Meier overall survival estimates were calculated and the log-rank test was used to compare overall survival in patients with and without HRA (stratified by the number of HRA). A multivariable-adjusted Cox regression model was used to assess the effect of HRA on overall survival adjusting for age, sex, International Staging System (ISS) stage, and first-line therapy (immunomodulator, proteasome inhibitor, upfront autologous hematopoietic stem cell transplantation). Patients diagnosed after 2014 (approximately 15% of the cohort) routinely underwent evaluation for gain(1q), therefore the hazard ratios represent conservative effect estimates. P-values below 0.05 were considered statistically significant.

**Results:** The median age at diagnosis was 65 years (28 - 95), 708 (60%) of the patients were male. There were 372 HRA in 327 patients (28% of the cohort): 170 (45%) del(17p), 110 (29%) t(4;14), 45 (12%) t(14;16), 8 (2%) t(14;20), and 42 (12%) gain(1q). Of the 280 patients with 1 HRA 130 (46%) had del(17p), 120 (43%) had a high-risk translocation, and 30 (11%) had gain(1q). Of the 46 patients with 2 HRA 34 (76%) had del(17p) and a high-risk translocation, 6 (13%) had a high-risk translocation and gain(1q), 5 (11%) had del(17p) and gain(1q), and 1 had 2 high-risk translocations. There was 1 patient with 3 HRA: del(17p) and t(4;14) and gain(1q). The median overall survival was 6.6 years (6.0 - 8.0) for the entire cohort (n=1181), 8.3 years (6.7 - 8.9) for those without HRA (n=854, 72%), 4.8 years (3.9 - 5.6) for those with one HRA (n=280, 24%), and 2.7 years (2.1 - 3.8) for those with 2 or more (2+) HRA (n=47, 4%). Figure 1 shows the Kaplan-Meier overall survival estimates stratified by the number of HRA (n=1181). The presence of 1 HRA (versus 0, HR 0.97, 95% CI 0.95 - 1.00, p=0.001, n=1181) and the presence of 2+ HRA (versus 1, HR 3.37, 95% CI 2.21 - 5.14, p<0.001, n=1181) were of prognostic significance after adjusting for age, sex, ISS stage, and first-line therapy. When adjusting for the revised ISS instead of the ISS the hazard was attenuated for 1 HRA (versus 0, HR 0.97, 95% CI 0.95 - 1.00, p=0.001, n=1087) and 2+ HRA (versus 1, HR 2.82, 95% CI 1.81 - 4.40, p<0.001, n=1087).

**Figure 1.**

**Summary/Conclusions:** Approximately 1 in 4 patients with newly diagnosed multiple myeloma presented with 1 HRA at the time of diagnosis, approximately 1 in 25 with 2 or more HRA. These patients experienced inferior overall survival suggesting a cumulative effect of multiple HRA.
## P332

**LENALIDOMIDE MAINTENANCE VS PLACEBO AFTER STEM CELL TRANSPLANT FOR PATIENTS WITH MULTIPLE MYELOMA: OVERALL SURVIVAL AND PROGRESSION-FREE SURVIVAL AFTER ADJUSTING FOR TREATMENT CROSSOVER IN CALGB**

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**Background:** At a prespecified interim analysis (December 2009), the phase 3 CALGB/ECOG 100104 (Alliance) study results surpassed the prespecified superiority boundary (significantly improved progression-free survival [PFS] for lenalidomide [LEN] maintenance vs placebo [PBO] after SCT) and the majority of PBO arm patients without progressive disease (PD) crossed over to LEN maintenance. An updated analysis (cutoff March 2015) showed significantly longer overall survival (OS) with LEN maintenance (HR, 0.56; 95% CI, 0.42-0.76). However, the crossover from PBO to LEN makes it difficult to assess the true treatment effect of LEN.

**Aims:** To examine the effect of LEN vs PBO on OS and PFS from randomization, adjusting for effects of crossover.

**Methods:** The rank-preserving structural failure time model (RPSFTM; Robins, *Commun Stat Theory Methods*, 1991) was used for crossover adjustment; the iterative parameter estimation (IPE; Branson, *Stat Med*, 2002) algorithm was used as validation. Survival was partitioned assuming a residual LEN effect after discontinuation. A landmark analysis was also performed at the Dec 2009 interim for patients who remained on treatment. Patients in the trial provided informed consent.

**Results:** Patients were randomized to LEN maintenance (n=231) and PBO (n=229) (intent-to-treat [ITT] population); 76 patients without PD crossed over from PBO to LEN. The median time from randomization to crossover was 11.5 months. The relative treatment effect for OS and PFS increased for LEN when adjusting for crossover using RPSFTM and IPE (Table 1). The landmark analysis at the Dec 2009 interim (PBO crossover, n=76; No crossover, n=154) showed the treatment effect is not dissimilar to the ITT analysis (HR 0.53; 95% CI, 0.25-0.76). Sensitivity analyses showed consistent results. Updated data will be presented at the meeting.

**Summary/Conclusions:** Adjusting for the potential diluting effects of crossover reduced median OS and PFS with PBO, and improved the treatment effect in the ITT analyses for OS and PFS for LEN vs PBO maintenance after SCT. The statistical significance of the ITT analyses was maintained throughout.

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>OS Median (95% CI)</th>
<th>PFS Median (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEN</td>
<td>62.9 (54.3-71.5)</td>
<td>17.6 (15.0-19.7)</td>
</tr>
<tr>
<td>PBO</td>
<td>50.1 (41.7-58.5)</td>
<td>11.6 (10.0-13.2)</td>
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**P334**

**EFFICACY AND SAFETY OF DARATUMUMAB, LENALIDOMIDE, AND DEXAMETHASONE VERSUS RRD ALONE IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA: UPDATED ANALYSIS OF POLLUX**


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**Background:** Daratumumab, a human monoclonal antibody targeting CD38, significantly prolongs progression-free survival (PFS) and achieves deep and durable responses when combined with other established standard-of-care regimens in patients with relapsed or refractory multiple myeloma (RRMM).

**Aims:** To provide updated efficacy and safety data from POLLUX, a multicenter, phase 3, randomized study of DRd versus Rd in RRMM.

**Methods:** Eligible patients with ≥1 prior line of therapy were randomly assigned to Rd (25 mg PO lenalidomide on Days 1-21 of each every-4-week [Q4W] cycle) or Rd (25 mg PO lenalidomide on Days 1-21 of each Q4W cycle) with or without daratumumab (16 mg/kg subcutaneously) on Days 1, 8, 15, 22, and 29 (28-day cycle) in the Rd group. Daratumumab was given (1.3 mg/m² intravenously or subcutaneously) on Days 1, 4, 8, 11, and 14, and dexamethasone (40 mg) on days 1, 2, 8, 15, and 22 (28-day cycle). KRD patients randomized to Rd with daratumumab in cycle 1 (1.3 mg/m² intravenously or subcutaneously) on Days 1, 4, 8, 11, and 14, and dexamethasone (20 mg/m²) on days 1 and 2 of cycle 1; 2 mg/m² thereafter; carfilzomib was omitted on days 8 and 9 in cycles 13–18. In ENDEAVOR, Kd patients received carfilzomib (20 mg/m² on days 1 and 2 of each cycle; 1 mg/m² thereafter) on days 1, 2, 8, 9, 15, and 16 and dexamethasone (20 mg) on days 1, 2, 8, 9, 15, 16, 22, and 23 (28-day cycle) in the Vd group. Carfilzomib was given (1.3 mg/m² intravenously or subcutaneously) on days 1, 4, 8, and 11, and dexamethasone (20 mg) on days 1, 2, 4, 5, 8, 9, 11, and 12 (21-day cycle). Comparisons were stratified by log-rank test; data presented here are per investigator assessment.

**Results:** In ASPIRE, 792 patients were randomized. Baseline characteristics were well balanced between the groups (median follow-up of 27 months). In ENDEAVOR, 929 patients were randomized. Baseline characteristics were well balanced between arms. At a median follow-up of 19.4 months (Kd) and 17.7 months (Vd), median PFS was 17.6 months (Kd) and 9.4 months (Vd) (HR: 0.53; 95% CI: 0.44–0.63; P <0.0001); 18-month PFS rates were 48.7% (Kd) and 23.9% (Vd) (HR: 0.62; 95% CI: 0.50–0.77; P <0.0001). Median time to next treatment (TTNT) was 26.1 months (Kd) and 14.5 months (Vd) (HR: 0.49; 95% CI: 0.40–0.60; P < 0.0001). 15.8% (Kd) and 14.9% (Vd) of patients discontinued because of adverse events (AEs). Grade ≥3 AE rates were 5.9% and 2.2% for hypertension, 3.9% and 1.8% for cardiac failure, and 4.6% and 5.4% for peripheral neuropathy for Kd and Rd, respectively. In ENDEAVOR, 929 patients were randomized. Baseline characteristics were well balanced between arms. At a median follow-up of 19.4 months (Kd) and 17.7 months (Vd), median PFS was 17.6 months (Kd) and 9.4 months (Vd) (HR: 0.53; 95% CI: 0.44–0.63; P <0.0001); 18-month PFS rates were 48.7% (Kd) and 23.9% (Vd) (HR: 0.62; 95% CI: 0.50–0.77; P <0.0001). Median TTNT was 26.1 months (Kd) and 14.5 months (Vd) (HR: 0.49; 95% CI: 0.40–0.60; P <0.0001). 15.8% (Kd) and 14.9% (Vd) of patients discontinued because of AEs. Grade ≥3 AE rates were 13.8% and 3.3% for hypertension, 5.2% and 2.0% for cardiac failure, and 2.4% and 8.6% for peripheral neuropathy for Kd and Vd, respectively.

**Summary/Conclusions:** Consistent with the primary analyses, these results show that incorporation of daratumumab into treatment regimens in patients with RRMM results in clinically meaningful improvements in PFS and a favourable benefit-risk profile.

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108 | haematologica | 2017; 102(s2)
narrow samples were collected, and minimal residual disease (MRD) was assessed at the time of suspected complete response (CR) and at 3 and 6 months after suspected CR at 3 different sensitivity thresholds (10⁻⁴, 10⁻⁵, and 10⁻⁶) using the ClonoSEQ™ next-generation sequencing-based assay (Adaptive Biotechnologies, Seattle, WA). Additional reflex testing using an anti-idio-
type antibody was used to confirm CRs in cases in which daratumumab inter-
ference with serum M-protein quantitation was suspected in patients with pos-
sible CR.

Results: Patients received a median (range) of 1 (1-11) prior lines of therapy; 55% of patients had received immunomodulatory agents (IMiDs), and 18% had been exposed to lenalidomide. After median follow-up of 17.3 months, DRD sig-
ificantly prolonged PFS compared with Rd alone (median: not reached vs 17.5
months; hazard ratio [HR], 0.37; 95% confidence interval [CI], 0.28-0.50; P<0.0001), with 18-month PFS rates of 76% and 49%, respectively. Responses
continued to deepen in the DRd group with longer follow-up, with significantly higher overall response rate (ORR; 93% vs 76%) and rates of very good partial response (VGPR) or better (78% vs 45%) and CR or better (46% vs 20%) with
DRd versus Rd alone (P=0.0001 for all), and MRD negativity was associated with prolonged PFS at 10⁻⁶ (Figure 1). Overall survival (OS) data are immature, with 40 (14%) deaths in the DRd group and 56 (20%) deaths in the Rd group (HR, 0.63; 95% CI, 0.42-0.95). Neutrophils
was the most common grade 3 or 4 treatment-emergent adverse event (53% with DRd vs 38% with Rd), and no new safety signals were reported with longer follow up. We will present updated efficacy and safety data based on approxi-
mately 23 months follow up at the meeting.

Figure 1.

Summary/Conclusions: DRD significantly improved outcomes compared with Rd alone, including PFS, ORR, depth of response, and MRD-negative rates, with a favorable safety profile that was maintained after longer follow up. These updated data continue to support the use of DRd in patients with RWM who received ≥1 prior therapy.

P335

DARATUMUMAB-BASED COMBINATION REGIMENS IN ELDERLY (≥75 YEARS) PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA: SUBGROUP ANALYSIS OF THE PHASE 3 CASTOR AND POLLUX STUDIES

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Background: Daratumumab (D) used in combination with bortezomb and dex-
amethasone (Vd; CASTOR) or lenalidomide and dexamethasone (Rd; POL-
LUX) significantly prolongs progression-free survival (PFS) with a manageable

Results: In CASTOR, 23/251 pts in the Vd group and 35/247 pts in the Vd
group were ≥75 years; the median (range) age for this group of pts was 78
(75-88) and 78 (75-85) years, respectively, with 100% and 94% with an ECOG
status ≤1. At a median follow-up of 13.0 months, discontinuation rates due
to treatment-emergent adverse events (TEAEs) were similar with Vd and Rd
(15% vs 20%). Common (≥10%) grade 3/4 TEAEs for Vd were thrombocy-
topenia (45% vs 37% with Vd), fatigue (15% vs 11%), pneumonia (15% vs
17%), and anemia (10% vs 11%). Infusion-related reactions (IRR) occurred
in 13 (65%) pts, with 10% having grade 3/4 IRR, but no pts discontinued due
to IRR. Median PFS was significantly prolonged with Vd versus Rd (not
reached [NR] vs 17.3 months; hazard ratio [HR], 0.27; 95% CI, 0.12-0.61; P=0.0007), consistent with the overall PFS observed in CAS-
TOR (Figure 1). Higher overall response rate (ORR; 95% vs 79%) and rates
of complete response (CR) or better (25% vs 3%) and very good partial
response (VGPR) or better (70% vs 18%) were achieved with Vd versus Rd,respectively, consistent with the overall population. In the POLLUX study,
29/286 pts in the DRd group and 35/283 pts in the Rd group were aged ≥75
years; the median (range) age for this group of pts was 77 (75-89) and 78
(75-87) years, respectively, with 86% and 91% with an ECOG status ≤1. At a
median follow-up of 17.3 months, 10% of pts in the DRd group and 11% in the
Rd group discontinued due to TEAEs. Common (≥10%) grade 3/4 TEAEs for
DRd were neutropenia (45% vs 31% with Rd), hypokalemia (14% vs 3%), and
pneumonia (10% vs 11%). D-associated IRR occurred in 12 (41%) pts in the
DRd group, with 4 (14%) pts having grade 3/4 IRR. No patient discontin-
ued DRD due to IRR. Median PFS was significantly prolonged with DRd
compared with Rd in the elderly subgroup (NR vs 11.4 months; HR, 0.19;
95% CI, 0.06-0.55; P=0.0007), consistent with the overall PFS observed in POLFUX (Figure 1). ORR was higher with DRd versus Rd (93% vs 77%), and rates of CR or better (52% vs 9%) and VGPR or better (72% vs 41%)
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Summary/Conclusions: The results in elderly pts were consistent with those
observed in the overall study populations in terms of efficacy. Rates of most
common grade 3/4 hematologic TEAEs observed in this elderly population were similar to that of the overall populations, and IRR were manageable. This subgroup analysis supports the addition of D to standard-of-care regimens in elderly pts with
RWM.
ALL ORAL COMBINATION OF IXAZOMIB PLUS THALIDOMIDE AND Dexamethasone FOR RELapsed OR refractory MULTIPLE MYELOMA: INTERIM DATA OF AN ONGOING PHASE II TRIAL


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Background: Ixazomib is a novel, effective oral proteasome inhibitor with a favorable toxicity profile. Recent studies showed significant activity as single agent with dexamethasone and in combination with other agents. The Tourmaline trial showed superior PFS with ixazomib plus lenalidomide and dexamethasone in pts with relapsed or refractory multiple myeloma (R/RMM).

Aims: Here, we evaluate the activity and tolerability of ixazomib plus thalidomide and dexamethasone (IxaThalDex) in pts with R/RMM.

Methods: Pts with R/RMM and one or more prior lines of therapy (TX) with the following criteria were eligible: Measurable disease, ECOG PS ≤2, ANC ≥1000/µL, platelet count ≥500000/µL, GFR ≥15ml/min. Treatment regimen: Ixazomib (4mg, d 1, 8 and 15), thalidomide (100mg/d), and dexamethasone (40mg d 1, 8, 15). Pts in 11/15 pts received 3 cycles and 5 pts were evaluable per protocol (PP). Full documentation of ≥2 cycles is available for 52 pts, with a median number of 4 cycles and a median FU of 7.4 mos. A PR or better was achieved in 33% of pts (28%, 18 grade 1 or 2, one grade 3). During the study, the incidence of hematological toxicity of grade ≥3 had anemia (23 grade 1/2 and 9 grade 3). Thrombocytopenia was recorded in 11 pts (14%, 4 grade 2 and 6 grade 3). The most frequent non-hematological toxicity was fatigue observed in 21 pts (32%) and infections noted in 27 pts (including 6 pts with pneumonia and 1 pt with sepsis). Polynuropathy was seen in 19 pts (28%, 18 grade 1 or 2, one grade 3). During the study, the incidence of new PNP was relatively low (17 new and two worsening PNP).

Summary/Conclusions: The all oral IxaThalDex regimen showed an ORR of 63% with no difference in pts with/without high-risk cytogenetics, a CBR of 67%, and a PFS of 10.4 mos in pts with R/RMM. The regimen was well tolerated and was associated with a low incidence of mainly grade ≤2 PNP, which required dose reduction in one patient only. Response rates improved with continuation of therapy and treatment was associated with an increase in health related QoL.

Figure 1.

EVALUATION OF GROWTH DIFFERENTIATION FACTOR-1 (GDF15) AS A NEW BIOMARKER FOR RENAL OUTCOMES IN DIFFERENT COHORTS OF PATIENTS WITH LIGHT CHAIN AMYLOIDOSIS

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Background: Growth differentiation factor-15 (GDF-15), is a member of the TGF-beta family, and is involved in several pathological conditions, including inflammation, cancer, cardiovascular, pulmonary and renal diseases. Serum GDF-15 levels add prognostic information to conventional prognostic factors, such as NT-proBNP and troponins, in cardiovascular disorders and has also shown to be associated with renal damage and risk of end stage renal disease in patients with diabetes. Increased serum GDF-15 levels have also shown to be correlated with early death and shorter survival independently of other cardiovascular biomarkers and Mayo stage. Because GDF-15 was also associated with renal outcomes we evaluated the prognostic value of GDF-15 levels in two independent cohorts of patients with AL amyloidosis and renal involvement who were treated in two different centers (Pavia Amyloidosis Center and Department of Clinical Therapeutics, Athens).

Aims: To evaluate the prognostic value of GDF-15 levels in independent cohorts of patients with AL amyloidosis and renal involvement.

Methods: Circulating levels of GDF-15 were measured by a novel pre-commercial immunoassay (R&D Diagnostics) in stored serum. The Pavia cohort included 135 and the Athens cohort included 76 patients with AL amyloidosis and renal involvement. Standard criteria were used for the diagnosis, evaluation of organ involvement and cardiobio marker-based risk stratification. Renal staging was based on the system proposed by Palladini et al., based on baseline laboratory investigations.

Results: Median age and involved FLC levels were similar between the two cohorts. However, heart involvement was more common in Pavia cohort (72% vs 53% p=0.005). Mayo stage disposition was also different (17%, 46% & 37% for stage 1, 2 & 3 in Pavia vs 30%, 43% & 27% in Athens cohort, p=0.08, but stage 3B was similar, 13% vs 12%). Also there were differences in peripheral nerve involvement (9% in Pavia vs 21% in Athens cohort, p=0.025). Median eGFR and renal stage distribution (26%, 54%, 20% vs 20%, 54%, 26% for renal stage 1-2 & 3 respectively) were similar between the two cohorts (p=0.544). Median follow up for the Pavia cohort was 18 months and for the Athens cohort was 45 months (p<0.001). Survival at 2 years was 59% for Pavia and 56% for Athens cohort. Median GDF-15 levels was 3454 pg/ml in Pavia (range 624 to >100000) and 4152 pg/ml (range 626 – 71475) in Athens cohort (p=0.09), while 93% and 94% of patients in the two cohorts had GDF-15 levels >1200 pg/ml (the upper limit of normal for individuals without cardiovascular disease). We then evaluated the prognostic significance regarding renal outcomes (dialysis): GDF-15 >4000 pg/ml was associated with a HR of 6 (95% CI 2 15.6, p=0.001) in Athens cohort (progression to dialysis within 2 years in 7% vs 47%); while, by applying the same cutoff in patients in Pavia cohort, 2-year dialysis rate was 10% vs 37% (HR: 3.95, 95% CI 1.6-15, p=0.004). Although renal stage discriminated 3 groups in univariate analysis in each cohort, in multivariate analysis, GDF-15 >4000 pg/ml outperformed renal stage by eGFR and proteinuria and was the only independent prognostic factor for progression to dialysis in each cohort (Figure 1).

Figure 1.

Figure 1.
Summary/Conclusions: Our study validated and confirmed in two independent cohorts, with differences in their characteristics, the prognostic value of GDF-15, which emerges as a novel biomarker with prognostic implications for different outcomes in patients with AL amyloidosis. Importantly, GDF-15 emerges as a strong biomarker for renal outcomes in patients with AL amyloidosis.

P338
AN OPEN-LABEL, PHASE 2 STUDY TO EVALUATE THE ORAL COMBINATION OF IZAXOMIB, CYCLOPHOSHAMIDE AND DEXAMETHASONE IN TRANSPLANT-INELIGIBLE PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA
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Background: Proteasome inhibitor (PI)-based combinations are standards of care in all lines of MM therapy. As the treatment paradigm moves to focus more on extended therapy, new combinations are needed that will be efficacious and tolerable, while giving pts the flexibility of taking their treatment at home. Combinations of ixazomib, the first oral PI, with immunomodulatory drugs (IMiDs) are feasible and effective; however, there may be pts for whom the use of IMiDs is not desirable. Therefore, triple combination of ixazomib with alkylators have been studied.

Aims: This phase 2 study (NCT02046070) evaluated the safety and efficacy of the all-oral ICd regimen in transplant-ineligible pts with NDMM. Primary endpoint was rate of CR+VGPR during induction. Secondary endpoints included tolerability and toxicity, overall response rate (CR+VGPR+PR) throughout treatment, time to response, PFS, and quality of life (QoL).

Methods: Adult pts with NDMM who were transplant-ineligible were randomized (1:1) to receive oral ixazomib 4.0 mg plus oral cyclophosphamide 300 mg/m² (Arm A) or 400 mg/m² (Arm B) on days 1, 8, 15, and dexamethasone 40 mg on days 1, 8, 15, and 22, for up to 13 x 28-day cycles as induction. Pts with ≥SD and an acceptable toxicity profile then received single-agent ixazomib maintenance therapy until PD, death, or unacceptable toxicity.

Results: 70 NDMM pts were enrolled (n=36 Arm A; n=34 Arm B): median age 73 years (range 61–87); 47% male; 31%/33%/29% ISS stage I/II/III MM; 50% had a cardiovascular/pulmonary comorbidity; 9% had high-risk cytogenetics (t(4;14), t(14;16), del 17p). At data cut-off (29 June 2016), pts had received a median of 19 cycles; 66% had completed 13 ICd induction cycles and proceeded to ixazomib maintenance therapy; 10% were ongoing on therapy, and 53% had discontinued due to PD (18%), toxicity (18%), patient withdrawal (3%), or other reasons (10%). Confirmed responses by investigator assessment are shown in the Table 1. Median time to first/best response across arms was 2/4 months. After a median follow-up of 17.8/18.5 months in Arm A/B, median PFS was not reached. Combined PFS at 12/18/24 months was 81%/66%/59% (24-month PFS 84%/56% for Arm A/B). In Arm A/B, 94%/100% reported AEs; 72%/74% reported grade ≥3 AEs; and 47%/56% reported SAEs. The most common grade ≥3 AEs were neutropenia (22% [11%]), anemia (19% [27%]), diarrhea, nausea, peripheral edema (each 18% [26%]), vomiting (15% [21%]), fatigue, and constipation (each 14% [10%]). The most common grade ≥3 hematologic AEs were neutropenia (22% [31%]), anemia (10% [14%]), lower respiratory tract and lung infections (9% [13%]), and supraventricular arrhythmias (5% [7%]). There were 5 on-study deaths, none considered related to treatment. QoL (by EORTC QLQ-C30; Global Health Status) was maintained from baseline during the study.

Table 1.

Summary/Conclusions: Based on this phase 2 study, ICd is an active treatment regimen for pts with NDMM who are ineligible for transplant. This trial captured a population of pts that was elderly and with multiple comorbidities. In this context, the results with ICd, an all-or-tripler including a PI and alkylator, provide evidence of clinical efficacy with a manageable safety profile. With a median follow-up of ~18 months, median PFS was not reached and outcomes appear comparable to other regimens in elderly transplant-ineligible pts with NDMM. The preferred cyclophosphamide dose for ICd phase 3 studies is 300 mg/m², based on the similar PFS, higher response rate, and numerically lower rate of AEs vs 400mg/m². Updated PFS results will be presented at the meeting.

P339
THE ORAL PROTEASOME INHIBITOR IZAXOMIB IN COMBINATION WITH MELPHALAN-PREDNISONE FOR PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA: PHASE 1/2 DOSE-ESCALATION STUDY
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Background: Bortezomib-MP is a standard-of-care regimen for elderly NDMM pts. Whereas bortezomib is administered IV or SC, ixazomib is an oral proteasome inhibitor with a safety profile amenable to extended dosing that is approved in the US and EU, in combination with lenalidomide-dexamethasone, for the treatment of MM pts who have received at least 1 prior therapy. Based on the demonstrated feasibility and efficacy of a proteasome inhibitor-MP combination, the all-oral ixazomib-MP (IMP) regimen was evaluated in elderly, transplant-ineligible NDMM pts.

Aims: Primary phase 1 objectives were to determine the safety, MTD, and recommended phase 2 dose (RP2D) of ixazomib in combination with MP. The primary phase 2 objective was to determine the rate of CR+VGPR; secondary objectives included PFS and OS.

Methods: In phase 1, pts were enrolled to 4 arms – Arm A: ixazomib 3.0–3.7 mg (days 1, 4, 8, 11, 22, 25, 29, 32) plus M 9 mg/m² and P 60 mg/m² (days 1–4) in 42-day cycles (max 9 cycles); Arm B: ixazomib 3.0–4.0 mg (days 1, 8, 15) plus M 6 mg/m² and P 60 mg/m² (days 1–4) in 28-day cycles (max 13 cycles); Arm C: ixazomib 3.0–4.0 mg (days 1, 8, 15, 22, 29) plus M 4.0 mg/m² (days 1, 8, 15, 22, 29) plus M 4.0 mg/m² (days 1, 8, 15, 22, 29) plus M 30 mg/m² (days 1–4) in 28-day cycles (max 9 cycles). In phase 2, an expansion cohort was enrolled at the RP2D. On all arms, after IMP induction, pts could receive maintenance with single-agent ixazomib (days 1, 8, 15; 28-day cycles).

Table 1.

Results: 61 pts were enrolled, 11, 34, 11, and 5 to Arms A, B, C, and D (median age 74 yrs; 31% ISS stage III, 56% creatinine clearance ≤60 mL/min). Among

haematologica | 2017; 102(s2) | 111.
38 DLT-evaluable pts in phase 1, 10 had DLTs of Gr 3 rash (n=2, Arm A), Gr 3-4 thrombocytopenia (n=4, 1 pt in each arm), Gr 3-4 neutropenia (n=1, Arm A; n=4, Arm C, n=1, Arm D), Gr 4 hemorrhagic oesophageal ulcer (n=1, Arm B), Gr 3 ileus/neurogenic bladder (n=1, Arm B), Gr 3 vomiting/diarrhea (n=1, Arm B), and Gr 3 respiratory infection (n=1, Arm C). The RP2D was ixazomib 4.0 mg in Arm B, based on observed rates of toxicity; this cohort was expanded to 26 pts. Among all 61 pts, the median number of treatment cycles was 16; 36 pts (13 at RP2D) completed IMP induction and entered maintenance. Median number of maintenance cycles was 12. The maximum treatment duration was 1841 days (>5 yrs) at RP2D. Five pts remain on treatment (2 at RP2D); primary reasons for discontinuation were disease progression (48%) and adverse events (AEs, 21%). CR+VGPR rate was 43% (43% at RP2D), including 28% (22%) ≥CR and 19% (17%) sCR; median time to first response was 1.7 mos, and responses continued to mature over a long period (Table 1). Depth of response improved during ixazomib maintenance in 9/36 (25%) pts (VGPR to sCR in 5 pts; VGPR to CR in 2 pts; CR to sCR in 2 pts). Median TTP/PFS are shown in Table 1; median OS was not reached after median follow-up of 42.6/46.9 mos overall/at RP2D.

**Summary/Conclusions:** The RP2D was weekly ixazomib 4.0 mg plus M 6 mg/m² and P 60 mg/m² (days 1–4) in 28-day cycles, consistent with the ixazomib dose and schedule in TOURMALINE-MM1. AEs were mainly hematologic, infections, PN, and diarrhea. The all-oral IMP regimen is active in NDMM, with a 28% CR rate (19% sCR), a 43% ≥VGPR rate, and a median PFS of 23.5 mos; responses continued to improve over a prolonged period.

**Myeloma and other monoclonal gammopathies - Clinical 2**

**P340**

**FEASIBILITY AND EFFICACY OF DOSE ADJUSTED MELPHALAN – PREDNISONE – BORTEZOMIB IN PATIENTS ≥75 YEARS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA; PRELIMINARY RESULTS OF THE PHASE II HOVON 123 STUDY**


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**Background:** There is a high rate of toxicity-related discontinuation in elderly patients with NDMM, negatively affecting outcome. In order to predict feasibility of treatment the IMWG developed the frailty score based on age, (instrumental) Activities of Daily Living and the Charlson comorbidity index.

**Methods:** Patients were treated with 9 cycles of MPV: Mel 6 mg/m², day 1-4; Pred 30 mg/m², day 1-4; and Bort 1.3 mg/m² day 1,8,15 and 22 of a 35-day cycle. This first planned analysis was restricted to the first 140 consecutive patients out of 240 planned patients.

**Results:** Of the 139/140 eligible patients none were fit (because of age ≥75 years), 30/139 (22%) were unfit, 100/139 (72%) were frail, and 9/139 (6%) unknown. The median follow up was 17.0 months. The discontinuation rate of MPV in the total population was 42%; 27% in unfit and 46% in frail patients (p=0.09). When also patients were included who discontinued bortezomib only these numbers were 27% in unfit and 52% in frail (p=0.02). Importantly, 6 cycles of MPV were found to be feasible in 70% of patients, both in unfit (80%) and frail (69%) patients. Age >80 years was associated with a significantly higher discontinuation rate of MPV or bortezomib only (70% versus 35% in patients aged 75-80 years, p<0.01). WHO performance was not associated with discontinuation rate. Response on protocol was ≥PR 73%, ≥VGPR 38% and ≥CR 11%, not significantly different in unfit versus frail patients. Response after 6 cycles was ≥PR 68%, ≥VGPR 35% and ≥CR 2%. Median progression free survival (PFS) was 17 months: 20 for unfit and 16 months for frail patients (p=0.13). Overall survival at 18 months was 76%: 89% for unfit and 72% for frail patients (p=0.22). Frail patients were found to have significantly less grip strength and lower walking speed as compared to unfit patients (Table 1).

**Table 1.**

<table>
<thead>
<tr>
<th>Unfit patients n=30</th>
<th>Frail patients n=100</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (range)</strong></td>
<td>77 (75-80)</td>
</tr>
<tr>
<td><strong>WHO (%)</strong></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>17</td>
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<tr>
<td>III</td>
<td>8</td>
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<tr>
<td>IV</td>
<td>11</td>
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<tr>
<td><strong>IS (%)</strong></td>
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<tr>
<td>I</td>
<td>25</td>
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<tr>
<td>II</td>
<td>23</td>
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<tr>
<td>III</td>
<td>32</td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
</tr>
<tr>
<td>unknown</td>
<td>17</td>
</tr>
<tr>
<td><strong>Grip strength (kg)</strong></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>25</td>
</tr>
<tr>
<td>II</td>
<td>23</td>
</tr>
<tr>
<td>III</td>
<td>32</td>
</tr>
<tr>
<td>IV</td>
<td>11</td>
</tr>
</tbody>
</table>

However, 58% and 59% of frail patients had an intermediate or high walking speed and grip strength respectively. Vice versa, 8% of patients with low
walking speed and 12% of patients with low grip strength, were not frail. Therefore, functional assessments will hopefully be complementary to the IMWG frailty score in guiding future therapy in unfit and frail patients.

P341

THE EUROPEAN MYELOMA NETWORK EMN09 STUDY: CARFILZOMIB, BENDAMUSTINE, AND DEXAMETHASONE IS EFFICIENT AND SAFE IN PATIENTS WITH ADVANCED MULTIPLE MYELOMA

Background: Even after a prolonged treatment patients with multiple myeloma may have received little chemotherapy, however many may suffer from bortezomib-induced peripheral neuropathy (PN). Bendamustine (Benda) leads to increased levels of defective ribosomal products (DRiPs). Carfilzomib (Carf), a proteasome inhibitor not inducing PN, inhibits degradation of DRiPs leading to plasma cell apoptosis.

Aims: With this scientific rationale a CB combination of Carf and Benda and low dose dexamethasone (dex) was evaluated in a phase 1/2 trial in patients with relapsed/refractory multiple myeloma (RRMM).

Methods: Sixty-three patients with RRMM with ≥2 lines of prior therapy were enrolled with the last patient included in February 2017. Treatment consisted of 28-day cycles Benda 70 mg/m² on day 1 and 8, Carf was given on day 1, 2, 9, 15, 16 and 27 mg/m² after an initial dose of 20 mg/m². In 6 patients in the phase 1 part of the trial Carf was escalated to 36 mg/m². This was found to be tolerable.

Results: The phase 1 part of the trial suggested Carf at the 27 mg/m² level for the phase 2 part. Forty-one patients were evaluated for response and efficacy. After last data cut-off, the median follow-up was 5.95 months. Number of prior treatment lines ranged from 2 to 9, and ≥85% of patients had received previous transplantation, bortezomib and immunomodulatory drugs.

Summary/Conclusions: In this elderly RRMM patients treated late in their disease, the combination of CBd provides effective outpatient therapy. Neither nau- sea, hair loss nor PN were an issue. Although cardiopulmonary as well as vascular signs were not infrequent, the overall response rate was 42%. In 11 patients the median elapsed time from diagnosis to treatment start was 5.8 years. Forty-three percent of patients achieved at least a PR including 28% ≥VGPR and an overall benefit of 92%. Median progression-free survival was 11.4 months and the 1-year overall survival was 75%. Hematological toxicity was well manageable. Non-hematological adverse events ≥2 ≥3 of all transplanted pts, n=29; 2 patients have not fin- ished treatment yet, very good partial remission (PR) was achieved in 7 patients (24%) and partial remission (PR) in 7 patients (24%). Overall, 25 patients died. Cause of death was either progression of AL (N=16), sepsis (N=4), heart transplant rejection (n=3) or other (n=2). Patients that underwent HTx had a median survival of 46 months (2-177, 1-year survival: 77%).

P342

CHEMOTHERAPY BEFORE AND AFTER HEART TRANSPLANTATION FOR PATIENTS WITH ADVANCED CARDIAC AL MYELOIDOSIS

Background: Survival rates for patients with light-chain (AL) amyloidosis are greatly reduced by advanced cardiac involvement at Mayo cardiac stage IV with a median survival of 6.5 months. High-dose Melphalan (HDM) and autol ogous stem cell transplantation (ASCT) or other intensive chemotherapy regimens cannot be applied to those patients due to the high risk of therapy-related morbidity. One approach to improve the catastrophic prognosis of those patients is to perform a heart transplantation followed by intense chemotherapy.

Aims: Our aim was to examine the cases of cardiac AL patients treated with heart transplantation (HTx) at our center and to evaluate the clinical outcome of this treatment approach.

Methods: Data from 41 patients (21m, 20f) suffering from cardiac AL who were treated in our hospital between 2002 and 2017 were retrospectively analyzed. All patients were high-urgency listed for orthotopic HTx due to poor perspective of survival. Until 2009, 10 (24%) patients were listed, 8 of them with stage IV (multiple organ involvement). Thereafter, we excluded patients with multiple organ involvement. All data are derived as medians with range or absolute numbers. Survival curves were calculated using the Kaplan-Meier method.

Results: Median age was 51 years (35-63) at diagnosis. Amyloidogenic lambda light-chains (LC) were detected in 39 and kappa light-chains in 4 patients. Median dFLC was 331 (69 – 2752) and median plasma cells in bone marrow were 13% (5-35). Median NT-proBNP was 6.332 ng/l (1.500 -5194), median cTNT 0.11 µg/l (0.01- 0.52) and median hsTNT was 60 ng/l (28-448) at diagnosis. Median NYHA stage was 3 (2-3) and median MADO 2004 stage was 3 (2-3). Serum creatinine was at 1.4 mg/dl (0.6-2.4), proteinuria at 0.1 g/day (range 0-10.7). Patients stayed on the high-urgency waiting list for a median of 26 (range 3-54) before 2009, and a median of 64 days (8-259) after 2009. 35 patients were treated with chemotherapy prior to HTx (mostly deixa w/o Bortezomib) to reduce dFLC during the waiting time. Eight patients died before receiving HTx with a median survival (start point: HU listing) of 26 days (8-177). With a median of 5 months after HTx (4-29), 18 patients received ASCT. HDM was used with either 200 mg/m² (N=10) or reduced dosage (N=8) in patients with reduced kidney function (mostly due to renal complications after HTx). Complete remission (CR) was achieved in 7 patients (24% of all transplanted pts, n=29; 2 patients have not fin- ished treatment yet, very good partial remission (PR) was achieved in 7 patients (24%) and partial remission (PR) in 7 patients (24%). Overall, 25 patients died. Cause of death was either progression of AL (N=16), sepsis (N=4), heart transplant rejection (n=3) or other (n=2). Patients that underwent HTx had a median survival of 46 months (2-177, 1-year survival: 77%).

Summary/Conclusions: HTx followed by chemotherapy is a feasible treatment approach in patients with advanced cardiac amyloidosis. Patients who reach HTx have a nearly 50% chance for a very good hematologic remission (VGPR or better) and consecutively a favorable survival probability with a median OS of nearly 6 years in our series.
or unacceptable toxicity. Supportive care was allowed; thromboprophylaxis was required for all pts on hemodialysis. The primary endpoint was overall response rate (ORR). Key secondary endpoints included safety, renal response, time to myeloma response, time to renal response, duration of response, progression-free survival (PFS), time to progression, and overall survival (OS). All pts provided informed consent.

Results: Enrolment has been completed with 81 pts (33 in cohort A; 34 in cohort B; 14 in cohort C), of which 13 (16.0%) were still on treatment as of January 28, 2017. Median follow-up for OS was 7.8 months. A total of 68 pts (84.0%) discontinued treatment; 39 (48.1%) due to PD. Median age was 72 yrs (range, 52-86 yrs). 60.5% of pts were male, and median time from diagnosis was 3.8 yrs (range, 0.03-19.44 yrs). Pts received a median of 4 (range, 1-10) prior anti-myeloma therapies. All pts had prior treatment with LEN (100%) and nearly all with BORT (97.5%). Median relative dose intensity of POM was 0.94 in both cohorts A and B, and 0.99 in cohort C. ORR was 39.4%, 29.4%, and 14.3% in cohorts A, B, and C respectively. PFS and OS results are presented in the Table 1. Grade 3/4 anemia and thrombocytopenia occurred more frequently in cohort C, likely due to severe RI requiring dialysis (Table 1). AEs leading to dose reductions were 18.2%, 14.7%, and 14.3% in cohorts A, B, and C, respectively.

Table 1.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Grade 3/4 AEs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>18.2%</td>
</tr>
<tr>
<td>B</td>
<td>14.7%</td>
</tr>
<tr>
<td>C</td>
<td>14.3%</td>
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</tbody>
</table>

Summary/Conclusions: POM+LoDEX is efficacious in pts with RRMM with moderate or severe RI, including those on hemodialysis, who had more advanced disease due to worse renal function. The safety profile was acceptable among the three groups and no new safety signals were observed. This study demonstrates that POM+LoDEX can be administered in pts with moderate or severe RI, including those on hemodialysis.

Aims: To determine the safety, tolerability, and antitumor activity of pembrolizumab monotherapy in patients with RRMM.

Methods: Patients with RRMM who have failed ≥2 prior lines of therapy including a proteasome inhibitor and immunomodulatory drug (IMiD) received pembrolizumab 10 mg/kg every 2 weeks or 200 mg fixed dose every 3 weeks. Primary endpoints were safety, tolerability, and objective response rate (ORR) as determined by investigators, per International Myeloma Working Group 2006 criteria.

Results: At date cutoff of January 2, 2017, 30 patients were treated. The median (range) duration of follow-up was 15 (1-32) months. 28 (93%) patients discontinued the study; the most common reason was disease progression in 14 (47%) patients and clinical progression in 9 (30%) patients. 2 (7%) patients are still on treatment. Median (range) age was 70 (56-81) years. 21 (70%) patients had an ECOG performance status of 0. Patients received a median (range) of 4 (2-10) prior lines of therapy. 20 (67%) patients were lenalidomide refractory, 10 (33%) were double-refractory, 9 (30%) were triple refractory, and 1 (3%) patient was quadruple refractory. Among patients with received pembrolizumab at 10 mg/kg, the median (range) of pembrolizumab exposure was 6 (2-15) cycles; among those who received 200-mg fixed dose of pembrolizumab, the exposure was 3 (2-6) cycles. No patient experienced a response. Seventeen (57%; 95% CI, 37-75%) patients had stable disease. 13 (43%; 95% CI, 26-63%) patients had progressive disease as their best response. Treatment-related adverse events (TRAEs) occurred in 12 (40%) patients. The most common TRAE was anemia (n=5; 17%). arthralgia, asparatate aminotransferase increased, fatigue, hyperglycemia, hypothyroidism, myalgia, pruritus, and blurred vision occurred in 1 patient each. A grade 3 TRAE (myalgia) occurred in 1 (3%) patient. There were no grade 4 TRAEs or deaths. A grade 3 TRAE, 1 (3%) patient had an immune-related adverse event (grade 1 pruritus).

Summary/Conclusions: The safety profile of pembrolizumab in RRMM was consistent with that observed with other cancers. Best response observed while on pembrolizumab monotherapy was stable disease. Recent results of ongoing studies, such as KEYNOTE-023 (NCT02036802), demonstrate promising efficacy of pembrolizumab in combination with IMiDs (lenalidomide) and dexamethasone in patients with RRMM.

P345

ASSESSMENT OF MOBILIZATION COST FOR MULTIPLE MYELOMA USING 2 DIFFERENT STRATEGIES: HIGH-DOSE CYCLOPHOSPHAMIDE VERSUS PLERIXAFOR. ON BEHALF OF IFM


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Background: Treatment with autologous transplantation (ASCT) remains the standard of care upfront for Multiple Myeloma patients considered eligible for transplant. Peripheral blood stem cell (PBSC) collection, also called mobilisation, is needed prior to ASCT. The optimal methodology for mobilizing PBSC has yet to be defined, with either G-CSF alone, also called steady state procedure, or use of Plerixafor, a CXCR4 antagonist (Mozobil®)+G-CSF or high dose cyclophosphamide (excluding direct medical costs) versus direct medical costs.

Methods: This is an observational cohort database analysis of 112 consecutive patients with MM treated upfront with ASCT between 2009 and 2013 and that had been mobilized with either high dose cyclophosphamide or plerixafor from 15 IFM centers. Patients must have successfully underwent ASCT. This study was not aimed at evaluating the suitability or advisability of one therapy versus another. A cost-consequences analysis of the different regimens of mobilization was performed. VERSUS PLERIXAFOR. ON BEHALF OF IFM

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Aims: To look at all skeletal surveys requested across 3 large hospitals in UK over a year and analyze their justification, effectiveness and utility. To decide if a rational clinic–biochemical algorithm could be used to reduce the number of imaging requests, thereby avoiding unnecessary radiation exposure and make a possible switch to modern imaging methods cost effective.

Methods: A total of 397 skeletal surveys were performed across three hospitals over one year. The data set was analyzed for clinical indications, paraprotein level, rationale for requesting the skeletal survey, the diagnostic yield and also the number of follow up CT/PET or MRI required. A pragmatic algorithm was developed and applied to see if the requests were justified and could have been safely reduced. (Figure 1).

Results: Of the 397 analyzable skeletal surveys performed, 266 were on myeloma, 81 for MUGS, 48 were for non-paraprotein related indications. Of the 266 myelomas, 30% of skeletal surveys were reported as positive according to IMWG criteria. A detailed analysis of 130 myeloma patients revealed a significant proportion of false negatives (6%) and false positives (7%), highlighting the insensitivity and poor specificity of this imaging modality. More importantly more than a third (38%) of myeloma patients required follow up imaging with MRI, PET or WBLC, irrespective of the initial skeletal survey result, indicating a significant duplication rate and waste of resources. In the MUGS group, majority of skeletal surveys were negative (91%) but 9% were reported as positive. Follow up imaging with CT and MRI was performed in 23% of the MUGS group. However none these were positive. When the clinic-biochemical algorithm was applied, the number of requests was reduced by at least a quarter (24%), avoiding unnecessary radiation exposure and precious resources.

Summary/Conclusions: - Skeletal survey has very limited role in investigation of paraproteinaemia and should be abandoned. - Our pragmatic clinic-biochemical imaging algorithm reduced imaging requests significantly (24%) allowing the preferred imaging modalities to be performed productively in a cost effective way in face of ever increasing health care cost and demands.
Background: In multiple myeloma (MM), abnormal serum free light chain ratios (FLCr) after therapy associate with poor prognosis, independent of depth of response. However the value of FLCr in the context of minimal residual disease (MRD) remains unclear. A proportion of MRD-negative patients experience early relapses and conversely, some MRD-positive patients can endure long-term survival; which may result from improved immunosurveillance following normal plasma-cell recovery. Aims: We hypothesised that serum FLCr levels and ratios add clinical value at the time of MRD assessment. Methods: The study included 275 intact immunoglobulin MM patients from the IFM2009 clinical trial who achieved at least a very good partial response (VGPR) after consolidation therapy. Median PFS from the end of consolidation therapy was 38.3 months; median OS was not reached. Serum FLCs were measured using multiple analytes and immunoassays (The Binding Site). Normal range for k/l FLCr was 0.26-1.25. We defined immunosuppression as levels of both the uninvolved (polyclonal) FLC+uninvolved heavy+light chain (HLC; measured with Heylityle) below their normal range. MRD assessment in bone marrow samples was based on 4-colour multiparametric flow cytometry (MFC).

Results: At the end of consolidation, 79/275 (29%) patients were MRD-positive; 79/275 (29%) had abnormal FLCr, 16/275 (6%) had elevated iFLC, with immunosuppression identified in 52/275 (19%). Using Cox regression all the variables associated with shorter PFS (p<0.001 for all) and OS (p<0.050 for all; except elevated iFLC which showed a trend towards shorter OS (p=0.070)). Among the 363 MRD-negative patients, 37/196 (19%) had abnormal FLCr. 2/196 (1%) had elevated iFLC with immunosuppression identified in 23/196 (12%). Median PFS for MRD-negative patients was not reached; however both an abnormal FLCr (median PFS: 31.4 months; p<0.001) and immunosuppression (median PFS: 31.4 months; p=0.005) identified a group of patients with poorer outcomes. On the other hand, median PFS for MRD-positive patients was 21.3 months; 42(53%) of these patients had abnormal FLCr and dismal outcomes (median PFS 12.6 vs 30.7 months for abnormal vs normal FLCr, respectively; p=0.004). Absolute FLC measurements did not reach statistical significance for PFS in these patients.

Summary/Conclusions: Serum FLC measurements in combination with low-sensitivity MFC bone marrow assessment at the end of consolidation therapy render the most powerful prognostic information in MM patients achieving deep responses. In those where disease is no longer detected using MFC, abnormal FLCr confer poor prognosis, which may partly be due to inefficient immune recovery. Absolute FLC measurements were not informative, supporting the rationale of evaluating biomarkers of the tumour and immune system recovery. Our results warrant further studies to validate the clinical utility of FLC measurements in combination with next-generation (8-colours) flow cytometry.
MYELOPROLIFERATIVE NEOPLASMS - CLINICAL 1

P350

RAS-PATHWAY MUTATION PATTERNS DEFINE EPIGENETIC SUBCLASSES IN JUVENILE MYELOMONOCYTIC LEUKEMIA


Aims: Genetic events in these cases identified additional gene alterations leading to frequent co-occurrence of 2 mutations activating the RAS-RAF-MEK-ERK pathway in the HM and IM subgroups. This finding was paralleled by a significant up-regulation of DNMT1 and DNMT3B expression suggesting aberrant activation of the DNA methylation machinery in this context.

Summary/Conclusions: Our integrated approach identified three JMML subgroups characterized by distinct clinical and biological features. We provide evidence for a molecular mechanism by which additional mutations are presumably further activating the RAS-RAF-MEK-ERK pathway, mediate DNA hypermethylation via up-regulation of DNMTs in more aggressive JMML cases.

P351

CYTOGENETIC ABNORMALITIES IN PRIMARY POLYCYTHEMIA VERA AND SECONDARY MYELOFIBROSIS: CORRELATION WITH GENETIC ALTERATIONS AND PHENOTYPE IN THE MYSEC STUDY


Aims: We hypothesized that DNA methylation profiling, either alone or in combination with genetic alterations, might provide a molecular basis for disease classification.

Methods: Genome-wide DNA methylation analysis using the HumanMethylation450 BeadChip array was performed in a discovery cohort of 20 JMML patients. We developed a strategy to eliminate methylation events that attribute fail to fully represent the observed disease heterogeneity.

Results: Systematic DNA methylation analysis of JMML samples identified three subgroups with low, intermediate and high methylation levels (LM, IM, and HM). Detailed analysis of the validation cohort not excluding the Noonan patients identified an association of methylation groups with clinical features.

Background: Myeloproliferative neoplasms (MPN) are characterized by clonal hematopoiesis that results in the development of myeloproliferative diseases. JMML is an aggressive myeloproliferative disorder of early childhood. While some cases show spontaneous remission, allogeneic hematopoietic stem cell transplantation (HSCT) remains the only curative treatment option for the majority of patients, however, the 5-year event-free survival rates remain only about 50%. Hyperactive RAS signaling is assumed to be the main driving event in JMML. It is caused by genetic alterations in CBL, KRAS, NF1, NRAS, or PTPN11 in about 90% of patients. So far, there is no clear understanding of how RAS pathway mutations relate to the heterogeneous disease biology and variable clinical outcome seen in JMML patients. As a consequence, established clinical and genetic markers fail to accurately predict the response to allogeneic transplantation.
was 70 years (21-89). Median (range) values for leukocytes, neutrophils, hemoglobin, platelets and bone marrow blasts at the time of sample collection for sequencing were 13.4 (1-179) x 10^9/L, 7.9 (0.4-152.4) x 10^9/L, 9.1 (3.1-15) g/dL, 123 (6-1168) x 10^9/L and 2% (0-17), respectively. On univariate analysis (n=97), only the presence of EZH2 and ZRSR2 mutations were associated with trends towards statistical significance for survival. Mutated EZH2 adversely impacted survival (p=0.06), and mutated ZRSR2 was associated with a non-significant improvement on survival (p=0.074). The IPSS-R for MDS was useful to differentiate between risk groups with different survival times (p=0.065) while the dynamic IPSS for PMF (Passamonti et al. Blood 2010) was not (p=0.39). On multivariate analysis, only EZH2 mutations and IPSS-R very low risk (versus all other categories combined) were statistically significantly associated with inferior and superior survival, respectively.

Summary/Conclusions: In this cohort of 97 patients with WHO-defined MDS/MPN-U, mutations in genes encoding epigenetic regulators (e.g., TET2, ASXL1, EZH2), spliceosome components (e.g., SRSF2, SF3B1, ZRSR2, U2AF1) or signaling molecules (e.g., KRAS, NRAS) that are commonly mutated in AML and SETBP1 were found at frequencies ≥10%. Although the analysis is limited by small numbers, EZH2 mutations were independently associated with poor survival. This represents the largest cohort of patients with MDS/MPN-U interrogated for mutations in multiple genes to date.

P353 MUTATIONAL LANDSCAPE OF MYELODYSPLASTIC SYNDROME/ MYELOPROLIFERATIVE NEOPLASM - UNCLASSIFIABLE

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Background: MDS/MPN-U is a rare, poorly characterized myeloid neoplasm within the MDS/MPN category in the World Health Organization (WHO) classification. A median survival of 12.4 months from time of referral was previously reported for a cohort of 85 patients with MDS/MPN-U seen at the MD Anderson Cancer Center (MDACC, DiNardo et al. Leukemia 2014). The International Prognostic Scoring System (IPSS) for MDS (Greenberg et al. Blood 1997) discriminated amongst prognostically distinct categories in that cohort, whereas neither the IPSS for primary myelofibrosis (PMF, Cervantes et al. Blood 2009) nor the revised IPSS (IPSS-R) for MDS (Greenberg et al. Blood 2012) did. Median survival of 21.4 months from time of diagnosis was reported in a multi-institutional cohort (n=69, Wang et al. Blood 2014). Information on the genomic landscape of MDS/MPN-U is limited to one report on the frequency of SETBP1 mutations (8.3%, Meggendorfer et al. Leukemia 2013).

Aims: To describe the mutational landscape of MDS/MPN-U using targeted multi-gene sequencing.

Methods: Targeted sequencing was performed on DNA from 97 patients with MDS/MPN-U (diagnosed per WHO 2008 criteria but excluding refractory anemia with ringed sideroblasts and thrombocytosis) seen across 4 US institutions (MDACC, 43; Cleveland Clinic, 29; Moffit Cancer Center, 16; Vanderbilt University, 9). Gene panels were varied between institutions, with 20 genes (ASXL1, CBL, DNMT3A, ETV6, EZH2, IDH1, IDH2, JAK2, KIT, NPM1, NRAS, PHF6, RUNX1, SETBP1, SF3B1, SRSF2, TET2, TP53, U2AF1, ZRSR2) in common.

Results: Mutational frequencies for the 20 genes tested in all 97 patients were as follows: TET2, 28%; ASXL1, 27%; JAK2, 25%; SRSF2, 22%; EZH2, 15%; SF3B1, 12%; RUNX1, 12%; ZRSR2, 11%; SETBP1, 11%; U2AF1, 11%; NRAS, 10%; DNMT3A, 9%; TP53, 8%; CBL, 4%; ETV6, 4%; NPM1, 4%; IDH2, 2%; KIT, 2%; PHF6, 1% and IDH1, 0%. In addition, the frequency of mutations in ten other genes of interest in hematologic malignancies was assessed: BRAF, 0% (n=52); CSF3R, 4% (n=52); CALR, 4% (n=53); MPL, 3% (n=86); MLL, 1% (n=93); TET2, 0% (n=72); CEBPA, 4% (n=73); KRAS, 4% (n=81); PTPN11, 4% (n=82); and FLT3, 2% (n=82). Median survival for the whole cohort (n=97) was 12.4 months (range, 1-173). The 43 MDACC patients in this analysis were included in the cohort of 85 previously reported by DiNardo et al. Median age
LEUKEMIC TRANSFORMATION OF MYELOPROLIFERATIVE NEOPLASMS: IS NGS PROFILE THE BEST PROGNOSTIC BiomARKER?
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Background: Leukemic transformation occurs in 8-23% of myelofibrosis patients in the first 10 years after diagnosis and in 4-8% of polycythemia vera and essential thrombocythemia patients within 18 years of diagnosis and is almost always fatal.

Aims: We retrospectively analyzed the survival outcome of patients with myeloproliferative neoplasms (MPNs) who progressed to acute myeloid leukemia (AML) based on the treatments received, response, different prognostic groups according to the (ELN) and based on a next-generation DNA sequencing profile (NGS).

Methods: A total of 72 patients diagnosed in our institute with IMA secondary to MPNs between 2000 and 2016 were retrospectively analyzed. NGS was performed in 44 mutations. Results found by NGS were classified according three different cellular functions of interest (Tumors suppressor (TP53), ADN/Histones’ epigenetic (DNMT3A, EZH2,HD1/Z,ASXL1) and alternative splicing (SRSF2, U2AF1, ZRS2, PRPF8, SF3B1)) and three groups were determined: Group A: patients without altered cellular function; Group B: patient with one altered function; Group C: patients with more than one altered functions. AML treatment response was evaluated according to the previous proposed criteria for response assessment of AML secondary to MPNs. Overall survival (OS) was calculated according the different treatments, treatment response and NGS profiles.

Results: 72 patients who developed AML secondary to MPNs were included in the study. 63.9% (N=31) had prior ET, 25% (N=18) PV, 14.8% (N=17) PMF and comparison to the general population was not significant. The death rate of MPNs diagnosis was 108 months (range: 2.4-408). The median age at AML transformation was 70 (range: 38-89). The median time to AML transformation from MPNs diagnosis was 108 months (range: 2.4-408). Among these 72 AML, 5.6% (N=4) belonged to the favorable risk category according to ELN 2017. 13.9% (N=10) belonged to the intermediate risk category and 55.6% (N=40) to the adverse risk category. 45.8% (N=33) patients were treated with intensive chemotherapy (IC), 15.3% (N=11) with azacitidine (AZA) and 38.9% (N=28) with supportive care (BSC). Median OS was 4.5 months (range, 0.1-65), with no significant difference between the three ELN 2017 risk categories (respectively 2.5 months (range: 1-9), 5.5 months (range: 1-60) and 5 months (range: 1-36) in the favorable, intermediate and adverse risk categories). Patients who received IC (p<0.01) or AZA (p<0.05) had a significant better OS (median OS of 7 months (range: 0.5-65) and 8.5 months (range: 3-24) respectively) than patients who received BSC (median OS of 2 months, range: 0.1-36). However, there was no significant difference between the IC and HMA groups (p=0.44). 7 Patients in Complete Cytogenetic Response (CCR) or Acute Leukemia Response-Complete (ALR-C) received an alloSCT had a better median OS than the 9 patients who did not (23 vs 6.5 months, p=0.063). Patients with group A and B NGS profiles have a significant better median OS (respectively 14.8 and 9 months) than Group C (3 months) (p<0.05).

Summary/Conclusions: Our results confirm the poor outcome of patients with secondary AML treated with IC and suggest that AZA provides comparable OS.

Figure 1.

Figure 1. Summary/Conclusions: Patients with mastocytosis may have a significantly higher risk of developing a secondary non hematological cancer as compared to the matched general population. Favorable follow-up of these patients is warranted as the risk of malignancies may increase over time and reduce life expectancy.

INCIDENCE AND OUTCOME OF SECONDARY NON HEMATOLOGICAL CANCERS IN ADULT PATIENTS WITH MASTOCYTOSIS
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Background: Mastocytosis is a clonal disease characterized by heterogeneous manifestations and a normal life expectancy in the majority of cases. In such a condition, it is important to ascertain if other diseases, and particularly solid malignancies, can worsen the prognosis.

Aims: To assess incidence and outcome of secondary primary malignancies (SPM) in adult mastocytosis patients.

Methods: We performed a retrospective analysis of 826 adult (>18 years at diagnosis) mastocytosis patients diagnosed and regularly followed in 6 Italian Institutions. SPM were defined as de novo cancers diagnosed after mastocyto- sis. We excluded from the analysis non-melanoma skin cancers due to the possible under-reporting of such neoplasms by patients themselves. Also, we did not consider newly hematological neoplasms, as they mainly represent a progression from Systemic Mastocytosis (SM) to SM with an Associated Hema- tological Neoplasm (AHN). Standardized Incidence Ratio (SIR) was calculated as the ratio between the observed cases in our cohort and the expected cases in the sex- and age-matched general Italian population in the same time period (these data were retrieved from http://www.registrati-tumorit.it). Times to event (patient-years) were calculated from the diagnosis of mastocytosis to the date of SPM diagnosis, death, or last contact, whichever comes first. Survival curves were estimated according to the Kaplan-Meier method.

Results: Males were 450 (54%). Median age at diagnosis was 49.3 years (range 19-84). Median follow-up was 2.3 years (range 0-41). Subtype diagnoses were: Cutaneous Mastocytosis (n=46), Indolent SM (n=633), Smol- dering SM (n=10), SM-AHN (n=34), Aggressive SM (n=47) and Mast cell leukemia (n=2). Fifty-four patients were classified as having mastocytosis in the skin. Overall, 42 patients had a history of malignancies prior to the diagnosis of mastocytosis: in these patients we did not detect any relapse of their prior malignancy after the diagnosis of mastocytosis. A total of 35 SPM were diagnosed in 34 patients (4.1%). Median age at SPM was 56.4 years (range 36-74). The median time from diagnosis to SPM was 22 months. The overall rate of SPM was 12.8 per 1,000 person-years (95%CI: 9.1-17.6) while the rate in the general adult population was 7.6 per 1,000 person-years (95%CI: 7.5-7.7) resulting in an increased hazard ratio of 1.7 (95%CI: 1.2-2.3). The risk for SPM was higher than expected in females (SIR 1.93, 95%CI: 1.2-3.1) while it was not significantly increased in males (SIR 1.36, 95%CI: 0.2-2.2).

Using a genome-wide methylation analysis, patients with the lowest degree of DNA methylation within the most variable CpG sites experienced spontaneous resolution and only two (7%) experienced an event during follow-up.

Figure 1. Summary/Conclusions: Patients with mastocytosis may have a significantly higher risk of developing a secondary non hematological cancer as compared to the matched general population. Favorable follow-up of these patients is warranted as the rate of malignancies may increase over time and reduce life expectancy.
the diagnosis of prePMF have been added (anemia, leukocytosis >11 x10^9/L, palpable splenomegaly, increased LDH). For these reasons, standardization of morphologic findings in "true" essential thrombocythemia (ET) as these two entities have different clinical outcomes. The revised WHO criteria underscore the importance of distinguishing prefibrotic PMF (prePMF) from ET and prePMF diagnosed according to the new 2016 WHO criteria.

Aims: To explore the clinical course of patients with CSF3R-mutated CNL, and identify risk factor(s) associated with survival.

Methods: A retrospective study was conducted to assess natural history and identify risk factor(s) for survival in patients with CSF3R-mutated CNL. Survival analysis was performed by the Kaplan-Meier method taking the interval from the date of diagnosis to death or last contact. The log-rank test was used to compare survival data. Cox regression model was used for multivariable analysis.

Results: Data of 47 patients with CSF3R-mutated CNL were collected and analyzed. 35 (76%) patients were male. Median age was 62 years (range, 16-92 years). At diagnosis, 17 (36%) patients had fatigue, 2 (4%) had a fever, 8 (17%) experienced diarrhea or abdominal discomfort, 20 (43%) were asymptomatic and leukocytosis had been mostly an incidental laboratory finding. 20 (43%) patients had palpable splenomegaly, and 4 (9%), palpable hepatomegaly. PB parameters, median and (range), were WBC 42.4×10^9/L (14-120×10^9/L), hemoglobin 151 g/L (124-180 g/L), platelets 165×10^9/L (17-570), blast percentage 0% (0-10), neutrophil percentage 82% (70-99). The median of blast cells in bone marrow was 1% (range, 0-12%). 46 (98%) patients were in the chronic phase and 1 (2%) in the accelerated phase at diagnosis. Most of the CSF3R mutations was T618I (n=45, 96%), others were T568M (n=1, 2%), T552A (n=1, 2%), G617 (72.3%) patients and 41 (87.2%) patients were screened for ASXL1 and SETBP1 mutations, respectively. 21 (61.8%) patients harbored ASXL1 mutation and 22 (53.7%) harbored SETBP1 mutation. All patients were BCR-ABL1, PDGFR and FGR mutation negative, 2 were CALR mutation and JAK2V617F mutation positive, respectively. Hydroxyurea was the most frequently used therapy (n=48). Other therapies included interferon-a (n=7), hypomethylating agents (n=4), thalidomide (n=2), ruxolitinib (n=1), imatinib (n=3), dasatinib (n=1), chemotherapy (n=6), and transplant (n=2). With a median follow up of 17 months (range, 2-103 months), 7 patients progressed to blastic phase or acute myeloid leukemia (n=6) or myelodysplastic syndrome (n=1). 17 patients died. Survival rate at 30 months was 57%. Median survival was 39 months (95% CI 8.5-69.5). Multivariate analysis showed that WBC >40×10^9/L (HR=3.26, 95% CI 1.14-9.30, p=0.027) was the sole risk factor for survival. However, SETBP1 or ASXL1 mutation was not associated with survival.

Summary/Conclusions: High WBC count was independently predictive of shortened survival in patients with CSF3R-mutated CNL.

P357

CLINICAL PHENOTYPE AND OUTCOME OF ESSENTIAL THROMBOCYTHEMIA AND PREFIBROTIC MYELOFIBROSIS DIAGNOSED ACCORDING TO THE REVISED 2016 WHO DIAGNOSTIC CRITERIA

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Background: The World Health Organization (WHO) classification system for myeloid neoplasms was recently revised in 2016. The revised WHO criteria underscore the importance of distinguishing prefibrotic PMF (prePMF) from “true” essential thrombocythemia (ET) as these two entities have different clinical outcomes. For these reasons, standardization of morphologic findings in the bone marrow biopsy and an explicit definition of minor clinical criteria for the diagnosis of prePMF have been added (anemia, leukocytosis >11 x10^9/L, palpable splenomegaly and increased LDH). Aims: To compare the clinical phenotype at diagnosis and the outcome of ET and prePMF diagnosed according to the new 2016 WHO criteria. Methods: We identified in our database all patients affected with ET, prePMF and PMF diagnosed according to 2008 WHO criteria who satisfied these two requirements: a bone marrow fibrosis grade 0-1 at diagnosis and at least one DNA sample to define the mutational status. Firstly, the bone marrow morphology of all 404 identified patients was reviewed by an expert pathologist. Then, we reclassified patients according to the new 2016 WHO criteria as follows: patient with ET morphology were classified as ET, patients with PMF morphology and at least one clinical criteria (leukocytosis, anemia, increased LDH, splenomegaly) were classified as prePMF, patients with PMF morphology but without clinical criteria were classified as myeloproliferative neoplasms unclassifiable (MPNu).

Results: According to the new criteria our cohort included 269 patients with ET, 109 patients with prePMF and 26 with MPNu. By comparing clinical phenotype at diagnosis in prePMF, MPNu, and ET respectively, we observed that prePMF showed higher leukocyte count, lower hemoglobin levels, higher platelet count, higher LDH values, higher number of circulating CD34-positive cells, and showed more frequently splenomegaly (Table 1). The higher frequency of CALR mutations in prePMF compared to ET, while they did not differ in terms of thrombotic complications (cumulative incidence of thrombosis at 10 years 18.5% vs 18%, P=0.5). Finally, we analyzed the subgroup of “old” ET diagnosed according to 2008 WHO criteria. Of 358 “old” ET, 268 were reclassified as ET, 25 as MPNu and 65 as prePMF. The “old” ET reclassified as prePMF had a higher risk of overt myelofibrotic evolution compared to the “old” ET reclassified as ET (cumulative incidence of overt myelofibrosis at 10 years 9.7% vs 0%, P=0.03).
Background: The minimal effective treatment in Essential Thrombocythemia (ET) patients is tailored mainly on the basis of thrombotic risk scores (primarily non nocere). The Revised International Prognostic Score for Thrombosis in ET (R-IPSET-Th) is based on different combinations of Age >60 yrs (Age >60), JAK2 V617F mutation (JAK2+), and Prior Thrombosis (PrTh+).

Aims: To validate the R-IPSET-Th in a cohort of ET patients reclassified according to the WHO 2016 criteria.

Methods: The web-based Registro Italiano Trombocitemie (RIT) recruited since 2005 patients with thrombocythemic bcr/abl negative chronic myeloproliferative neoplasms (MPN). ET patients (reclassified according to WHO 2016 criteria) with complete information (characteristics at diagnosis, antiplatelet and/or cytoreductive treatment, date and description of thrombotic events during the follow-up) were considered for this analysis. According to the R-IPSET-Th score, the patients were divided in 4 thrombotic risk groups: Very Low Risk (VL: Age <60, absence of JAK2 mutations, no PrTh), Low Risk (LR: Age >60, Inter-Risk: IR: Age >60, High Risk (HR: PrTh+, or Age >60 with JAK2+). The Th-FUP/100 pt-yrs increased (p <0.01) as follows: 0.60%, 0.79%, 1.61%, and 1.91%, respectively. TFS progressively decreased (p <0.001) from VLR group to HR group (Figure 1). In detail, the probability of time from diagnosis to the first thrombosis) was determined for each risk group (Kaplan Meier analysis), and the curves were compared with the log-rank test.

The concordance between the R-IPSET-Th score and the IPSET-Th score was evaluated (Harrell C concordance index).

Results: Overall, 734 ET patients were analyzed (females 62%). Data at diagnosis were: Age >60 in 286 (39%), JAK2+ in 417 (57%), and PrTh in 126 (17%) patients. Moreover: CVRFs in 66%, PLT >1000 x 10^9/L in 17%, and WBC >10 x 10^9/L in 21% of patients. The patients in the 4 R-IPSET-Th score risk groups were: VLR 193 (26%), LR 197 (27%), IR 79 (11%), HR 265 (36%). The median follow-up was 12, 12, 9, and 11 years, respectively (whole cohort, 11 years). The rates of treatment were: 88%, 94%, 92%, 91%, respectively (whole cohort, 91%), with anti-platelet drugs (mainly low dose aspirin): 71%, 62%, 95%, 95%, respectively (whole cohort, 80%), with cytoreductive drugs (mainly hydroxy-carbamide). The Th-FUP were 103 (14.0%), with a rate increasing with the risk score (p <0.001): in VLR (n 15, 8%), in LR (n 20, 10%), and in HR (n 12, 15%), in HR (n 56, 21%). The Th-FUP/100 pt-yrs increased (p <0.01) as follows: 0.60%, 0.79%, 1.61%, and 1.91%, respectively. TFS progressively decreased (p <0.001) from VLR group to HR group (Figure 1). In detail, the probability of TFS was 0.98, 0.97, 0.94, 0.88 at 5 years, and 0.85, 0.87, 0.78, 0.54 at 20 years. The patient stratification according to the R-IPSET-Th and the IPSET-Th scores showed a concordance of 0.82 (Harrell C index).

Table 1.

Summary/Conclusions: In this study of the Registro Italiano Trombocitemie (RIT), we confirmed that the Revised International Prognostic Score for Thrombosis in ET (R-IPSET-Th) separated ET patients in 4 groups with increasing risk of thrombosis during the follow-up (p <0.001). According to the R-IPSET-Th score, an ET patient seems to have occurred in this cohort of ET patients (anti-platelets in almost all cases, and cytoreduction in 2/3 of VLR and LR cases), probably because other adjective risk factors have been considered.

Figure 1.

Summary/Conclusions: Myelofibrosis (MF) is characterized by significant inflammation driven by clonal dysregulation and subsequent disruption of cellular signaling cascades. Studies have confirmed a close relationship between circulating inflammatory BMKs and baseline symptom burden, along with the potential to mitigate symptoms upon improvement of these proteins (Dueck Blood, 2013). To date, no study has evaluated the correlations between elevated biomarkers (BMKs) and specific MF symptoms.

Methods: Biomarker levels at baseline, week 4 and 24 were measured along with MF symptoms (MFSAF 2.0-Mesa JCO 2013; collected during blinded phase of COMFORT-I). Patients were randomized to ruxolitinib vs placebo. BMKs were assessed using Rules-Based Medicine, Inc. (Austin, TX) Human MAP panel. Associations between the individual symptoms measured within the MFSAF and log2-transformed biomarker data were investigated at base-line using Spearman correlations. Mixed models were used to assess symptom and BMK changes over time. Models included terms for visit, arm, visit-by-arm interaction, age, sex, and body mass index (BMI).

Results: Study Population. A total of 309 subjects were randomized in COMFORT-I with median age of 68 (range 40-91). Approximately 46% of patients were female and 50% had primary myelofibrosis (61% high risk). All 309 subjects had BMKs measured at one or more of the three visits included in this analysis, with 308 having biomarker values paired with MFSAF symptom scores at the same visit. Correlations of Baseline Biomarkers and Symptoms. Total symptom score (TSS) statistically significantly (p<0.05) correlated with 20 BMKs at baseline (Table 1). For individual symptoms, spleen-related symptoms appeared to statistically significantly correlate more frequently with BMKs at baseline: abdominal discomfort (23 BMKs), feeling full (20 BMKs), and pain under left ribs (19 BMKs). Night sweats, itchiness, and bone or muscle pain significantly correlated with 15, 14, and 10 BMKs each. The BMKs with the strongest correlations (absolute Spearman correlation of at least 0.20 with p<0.001) with at least one symptom included APOA1, EPO, FERRITIN, MIP1A, and PSAF. Associations with Symptoms and Biomarker Change Over the Trial Course. Twenty BMKs were significantly associated with TSS over time. Like at baseline, BMKs appeared to be more often statistically significantly (p<0.05) associated with spleen-related symptoms over time including 25 and 24 BMKs for abdominal discomfort and feeling full, respectively. Night sweats, pain under left ribs, bone or muscle pain, and itchiness were associated with 20, 12, 12, and 9 BMKs each. Strongest associations (p<0.001) between symptoms and BMKs over time included VCA1 (4/6 symptoms+TSS), B2MCG (3/6 symptoms+TSS), LEPTIN (3/6 symptoms+TSS), TIMP1 (2/6 symptoms+TSS), TNFR1 (2/6 symptoms+TSS), INTLK18 (2/6 symptoms+TSS), and VWD (1/6 symptoms).
Platelet disorders: Basic

P360

NOVEL HETEROZYGOUS ITGB3 P.T746DEL MUTATION INDUCING SPONTANEOUS ACTIVATION OF INTEGRIN ΑΙΙΒ3 CAUSES AUTOSOMAL DOMINANT MACROTHROMBOCYTOPENIA WITH ABNORMAL ΑΙΙΒ3 LOCALIZATION

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Background: Congenital macrothrombocytopenia is a rare platelet disorder and its cause is genetically heterogeneous. Recently, integrin αIIb and β3 mutations have been identified in congenital macrothrombocytopenia patients with platelet aggregation dysfunction. Here, we found a novel, heterozygous ITGB3 mutation in a pedigree and examined how this mutation contributed congenital macrothrombocytopenia.

Aims: To detect gene mutations responsible for the congenital macrothrombocytopenia in this pedigree and reveal the molecular pathophysiology.

Methods: Whole exome sequencing (WES) was performed to detect gene mutations. Expression and activation state of αIIbβ3 in platelets were evaluated by flow cytometry (FCM) and western blotting (WB). The effects of mutations on αIIbβ3 activation state, phosphorylation of FAK, and morphological changes were analyzed in transfected cells by WB and immunofluorescence staining.

Results: The patients were 56-year-old Japanese woman and 2 of her 3 sons. They had no bleeding tendencies and near-normal bleeding time (Duke’s method). Hematological examination revealed their decreased platelet counts (58-86 x 10^9/l) with increase of mean platelet volume (12.8-14.5 fl). In all affected family members, giant platelets were observed on the peripheral blood smears. Platelet aggregation induced by ADP (1-10 µmol/l) and collagen (2 µg/ml) was obviously reduced although that induced by ristocetin (1.5 mg/ml) was within normal limit. The family pedigree indicates that the inheritance pattern is autosomal dominant. Common congenital macrothrombocytopenias, such as MYH9 disorders, Bernard-Soulier syndrome and type 2B von Willebrand disease were excluded by the absent leukocyte inclusion bodies, normal ristocetin-inducible platelet aggregation and normal platelet GPIb/IX expression, normal von Willebrand factor assays, respectively. WES revealed that all affected family members had a heterozygous ITGB3 p.T746del mutation. FCM showed decreased surface expression level of αIIbβ3 in the affected member’s platelets. However WB of platelet lysates showed that there was no difference in the total amount of αIIbβ3 among the affected and unaffected members and normal controls. FCM showed a constitutive activation of αIIbβ3 on the patient’s platelets as reflected by the spontaneous binding of PAC-1 antibody. Immunofluorescence staining using CHO cells showed membrane localization of αIIbβ3 in wild-type αIIbβ3-expressing cells and cytoplasmic localization in αIIbβ3 (p.T746del) expressing cells. Immunoblotting of αIIbβ3 (p.T746del) using αIIbβ3 antibody pointed out to spontaneous tyrosine phosphorylation of FAK and morphological changes, such as rhomboid changes, elongated changes, abnormal cytoplasmic protrusions, and membrane ruffling, in transfected cells. FAK inhibitor (1,2,4,5-Benzenetetraamine tetrahydrochloride) hindered the localization change of αIIbβ3 and the morphological changes, elongated changes, abnormal cytoplasmic protrusions, and membrane ruffling in transfected cells. FAK inhibitor (1,2,4,5-Benzenetetraamine tetrahydrochloride) hindered the localization change of αIIbβ3 and the morphological changes, elongated changes, abnormal cytoplasmic protrusions, and membrane ruffling in transfected cells. FAK inhibitor (1,2,4,5-Benzenetetraamine tetrahydrochloride) hindered the localization change of αIIbβ3 and the morphological changes, elongated changes, abnormal cytoplasmic protrusions, and membrane ruffling in transfected cells.

Summary/Conclusions: The autosomal dominant heterozygous ITGB3 p.T746del mutation was found to be responsible for constitutive activation of αIIbβ3 in the patients’ platelets as well as transfected cells. It is considered that ITGB3 p.T746del mutation unclasps the highly conserved membrane proximal complex of αIIb and β3 cytoplasmic tails and renders the activated form. Activation of αIIbβ3 leads to phosphorylation of FAK causing morphological changes in transfected cells, which is considered to reflect abnormal thrombopoiesis leading to the production of giant platelets. We conclude that platelet aggregation dysfunction is due to decrease of αIIbβ3 expression on the platelet membrane surface due to cytoplasmic localization. These results suggest that the gain-of-function mutation around membrane region of αIIbβ3 leads to macrothrombocytopenia with impaired surface αIIbβ3 expression.

Figure 1.

Summary/Conclusions: In ITP patients, ETP can induce overexpression of genes involved in platelet activation and megakaryopoiesis and also alter key/relevant/important-signaling pathways such as JAK/STAT and PI3K/Akt.

P361

CHANGES IN THE GENE EXPRESSION PROFILE OF IMMUNE THROMBOCYTOPENIA PATIENTS TREATED WITH ETPRODEGLOBULIN

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Background: Etlrombopag (ETP) is an orally bioavailable, small non-peptide molecule thrombopoietin receptor agonist that stimulates platelet production by a mechanism similar, but not identical to, endogenous thrombopoietin. ETP interacts with the transmembrane domain of thrombopoietin receptor, initiating a JAK/STAT signaling pathway inducing the proliferation and differentiation of the megakaryocytes to increase platelets production.

Aims: To assess the gene expression profile (GEP) and the underlying signaling pathways modified before and during the ETP treatment in chronic immune thrombocytopenia (ITP) patients.

Methods: 14 ITP patients (n=14) treated with ETP were evaluated. Complete response (CR) was defined as a platelet count of ≥100x10^3/mm^3 and treatment failure was defined as a platelet count of≤50x10^3/mm^3 for 4 consecutive weeks at the highest recommended dose of ETP, a major bleeding event, or the need to change therapy. RNA was isolated from mononucleated cells pre/post ETP treatment. The “paired” GEP of the ITP patients included the semi-supervised analysis cluster samples before and after (28 day) the treatment with ETP to detect changes attributed to ETP. This paired GEP was showed in Figure 1. The GEP workflow consisted of the following steps: 28-paired samples were hybridized to GeneChip® Human Gene 2.0 ST Array (Affymetrix®). The robust microarray analysis (RMA) algorithm was used for background correction and normalization, while signal expression was calculated by significance analysis of each microarray to provide a robust statistical inference by a permutation method. P-values were provided and adjusted by multiples testing using a false discovery rate (FDR). The pathways and upstream regulators related with the most differentially expressed genes were analyzed by in silico analysis tools: Advatica Bio’s PathwayGuide (http://www.advatica.com/pathwayguide) and DAVID Bioinformatics Resources.

Results: The median age of the 14 ITP patients enrolled in the study was 77 years (range, 35-87y), 64% patients (n=9) were treated with ETP after ≥2 lines of treatments. Only 3 patients were splenectomized. Median platelet (P) and white blood cell counts (WBC) increased after treated by ETP at day 28. (P and WBC pre: 14,15x10^3/mm^3 and 6,85x10^3/mm^3 vs P and WBC post: 132x10^3/mm^3 and 9,1x10^3/mm^3). All but two patients achieved CR (85.7%) and other 2 were considered failure of treatment. Regarding the gene expression profile, in silico analysis showed that the expression of 147 genes was modified after ETP treatment; all of them were overexpressed after treatment. Semi-supervised cluster analysis showed 2 groups: pre and post ETP treatment (Figure 1). Pathway analysis revealed that 38 genes were involved in the maintenance of hemostasis, most of them related to platelet activation (PTGS1, GP1BA or GP6). Interestingly, the paired GEP pointed out E2F1 and GFI1B as possible leaders of the increase of the megakaryopoiesis. Other signaling pathways overexpressed by ETP treatment are downstream routes of PI3K/Akt (GFI1B, JAM3, ITGB3 and ITGA2B) and platelet activation (GP6, GP9, GP1BA or PTGS1).

Figure 1.

Summary/Conclusions: In ITP patients, ETP can induce overexpression of genes involved in platelet activation and megakaryopoiesis and also alter key/relevant/important-signaling pathways such as JAK/STAT and PI3K/Akt.
antibodies against glycoprotein Iib (GPIib)IIa and/or GPIib/IX are considered to play a crucial role. B cell homeostasis and function are controlled by cell surface receptor-ligand interactions. The activation of PI3K is initiated by engagement of the pre-B cell receptor (BCR) and the BCR. The phosphatase and tensin homolog (PTEN) suppress the activity of the PI3K pathway. As a consequence, loss of PTEN function leads to excessive PI (3, 4, 5) P3 at the plasma membrane and to recruitment and activation of Akt family members that potently drive cell survival and proliferation. PTEN regulates normal signaling through the B cell receptor (BCR). In immune thrombocytopenia (ITP), enhanced BCR signaling contributes to increased B cell activity, but the role of PTEN in human ITP has remained unclear. Both IL-21/L-21R signaling and PI3K-PTEN molecules are involved in maintaining normal humoral immunity and deletion of autoreactive B cells. In this study, we want to determine whether abnormalities in PTEN might contribute to increase B cell responsiveness in this disease and IL-21 mediated PTEN induction was defective. Meanwhile, we want to evaluate the relation between the expression of PTEN in B cells and the prognosis of ITP, which will provide a theoretical basis of new treatment strategy for the ITP patient.

**Aims:** PTEN is involved in maintaining normal B cell function. Since B cell overactivity is characteristic of immune thrombocytopenia (ITP), we sought to determine whether abnormalities in PTEN might contribute to increase B cell responsiveness in this disease.

**Methods:** 1. This study recruited 28 newly-diagnosed CITP patients and 26 sex and age matched health volunteers as health controls (HC). Peripheric blood mononuclear cells were isolated from collected anti-coagulated blood. 2. Flow cytometry and real-time quantitative PCR were used for detecting the level of PTEN from PBMC cells of HC and CITP patients. 3. The relationship between PTEN levels and the disease severity of CITP was analyzed. 4. PBMC cells were incubated with human rIL-2 rIL-21 rCD40L or anti-IgM alone or in combination for 72h and after that the PTEN level was detected by flow cytometry. The proportion and surface activated marker of B cells were determined by flow cytometry.

**Results:** 1. Compared to HC the expression of PTEN was diminished in each CITP B cell population except IgD-CD38low/-memory B cells. In addition PTEN overexpression in normal B cells, followed by IL-21 and IL-2. Neither IL-21 alone nor CD40L plus anti-IgM nor the three in combination stimulated PTEN protein up-regulation in B cells in CITP patients. 2. ATRA, SEW2871 and extracellular S1P rescued the defect in PTEN expression of CITP patients (Figure 1).

**Summary/Conclusions:** There is a great discrepancy of PTEN expression in B cells in CITP patients (Figure 1).

**Figure 1.**

Aims: To determine whether the S1P levels in both the BM niche and within MKs, as well as S1PR expression of MKs contribute to the defective thrombopoiesis in ITP through impaired PPF.

**Methods:** The PPF of ITP-MKs was measured by an in vitro PPF assay using HSs from the BM. FACS (FITC; 1:20;4701-10). Additionally, all-trans-retinoic acid (ATRA), the S1pr1-specific agonist SEW2871, and extracellular S1P were used as interference factors. The concentration of S1P in the plasma and BM was analyzed by ELISA. The concentration of intracellular S1P was measured using liquid chromatography-mass spectrometry (LC/MS) analysis. Intracellular Sphk2, SFKs and cell-surface S1PRs were monitored using PCR and western blotting. The location of Sphk2 was analyzed by immunofluorescence using an anti-human Sphk2 antibody. The activities of Rac-GTP were quantified by pulldown assay.

**Results:** Significantly fewer numbers of proplatelet-forming MKs were observed in ITP cultures. The concentration of S1P in the plasma and in BM of patients with ITP was measured, of which the results showed no significant difference in the plasma/BM S1P ratio. Decreased expression of S1PR1 and S1P4 was observed in ITP-MKs. We found that downstream GiRac GTase signalling activated by S1PR1 was down-regulated. ITP-MKs exhibited decreased intracellular Sphk2, indicating less biosynthesis of intracellular S1P. Immunostaining of Sphk2 in ITP-MKs was performed, showing that Sphk2 was primarily localized to the nucleus of ITP-MKs. Intracellular S1P of ITP-MKs was further explored showing a decrease of megakaryocytic S1P production ascribed to significantly reduced SFK activity. ATRA, SEW2871 and extracellular S1P enhanced Rac-GTPase activity and SFP expression, which rescued the defect of PPF in ITP.

**Summary/Conclusions:** Decreased intracellular S1P ascribed to significantly reduced Sphk2, results in down-regulated SFK expression and activity, and decreased S1PR1 and S1PR4 down-regulate GiRac GTase pathway in ITP-MKs. Therefore, abnormal S1P/S1PR possibly plays a role in the pathogenesis of impaired PPF in ITP, which may be therapeutically regulated by ATRA.

**Figure 1.**

**Summary/Conclusions:** Immune thrombocytopenia B cell showed decreased levels of PTEN and the decrease was associated with low platelet count and positive serum platelet-specific antibody. The capacity of IL-21 to induce PTEN was absent in CITP. Together, these data suggesting that the defective PTEN expression, regulation and function contribute to B cell hyper-responsiveness in CITP.

**P363**

**DECREASED INTRACELLULAR S1P LEVEL AND S1P RECEPTORS EXPRESSED ON MEGAKARYOCYTES POSSIBLY CONTRIBUTE TO DEFECTIVE PROPLATELETS FORMATION IN IMMUNE THROMBOCYTOPENIA**

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**Background:** Immune thrombocytopenia (ITP) is a common autoimmune disorder characterized by increased bleeding tendency and isolated thrombocytopenia. The precise pathogenesis of the decreased thrombopoiesis in ITP remains unknown. It has been demonstrated that megakaryocytes (MKs) in ITP show impaired proplatelets formation (PPF) (Br J Haematol.2014;165:854-64). However, the pathogenesis of the impaired PPF in ITP is not entirely understood. Importantly, we found that CD40L and anti-IgM were the most potent inducers of PTEN to induce PTEN expression in B cells of HC was found by flow cytometry.

**Methods:** We determined whether abnormalities in PTEN might contribute to increase B cell responsiveness in this disease.

**Results:** We found that CD40L and anti-IgM were the most potent inducers of PTEN expression in B cells of HC was found by flow cytometry.
tin (PNA) that bind to galactose, N-acetyllactosamine and N-acetylgalac-
tosamine residues, respectively. The NOD/SCID mouse model was used to study the impact of different glycan patterns on the survival of human PLTs.

Results: In this work 37 sera from ITP patients and 25 sera from healthy donors were analyzed. In the LBA, after incubation with AAbs, different patterns of glycan modification were observed. 17/37 sera caused a significant increase in PLTs analyzed, and the serum level of thrombopoietin was normal or moderately (median fold increase: FI: 1.21, range: 1.08 - 1.40). 9/37 sera induced higher ECL binding (median FI: 1.02, range: 1.08 - 1.15). In contrast, 8/37 sera showed strong decrease in RCA binding (median FI: 0.52, range: 0.50 - 0.59). Sera from healthy donors did not induce significant change. Interestingly, not only GP-ib/IIa AAbs but also GPIIb/IIIa AAbs were able to modify glycan pattern. In NOD/SCID mice the administration of AAbs induced an accelerated clearance of human PLTs from the circulation. The destruction of human PLTs by ITP-AAbs was decreased but not completely prevented by a specific neuraminidase inhibitor that blocks glycan changes on PLT surface (survival of human PLTs after 5h: 48%, range 41-53% in 29%, range 22-29% in 49%).

Summary/Conclusions: Our results demonstrate that AAbs from ITP patients are able to induce cleavage of glycan moieties on the PLT surface in distinct manners. Antibody-mediated modification of glycan patterns seems to contribute to AAB-mediated PLT destruction.

P365
NOVEL RUNX1 MUTATIONS IN FAMILIES WITH INHERITED THROMBOCYTOPENIA
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Background: Familial platelet disorder with propensity to acute myeloid leukemia (FPD/AML) is a rare autosomal dominant inherited thrombocytopenia (IT) caused by mutations in the hematopoietic transcription factor RUNX1; an important hallmark of this IT is the increased risk of developing myeloid neoplasms, such as AML and myelodysplastic syndromes (MDS). FPD/AML is caused by different mutations of RUNX1 encoding the DNA binding subunit (known as core binding factor-alpha, CBF-alpha) of the CBF transcription complex. The N-terminus domain of RUNX1 (runt-homologous domain) mediates DNA binding and heterodimerization to CBF-beta, the other subunit of the CBF complex. The C-terminus of RUNX1 includes domains that are involved in transcription activation and repression. This IT is characterized by impaired megakaryopoiesis and moderate thrombocytopenia, with normal-sized and dysfunctional platelets.

Aims: To unravel the molecular basis of ITs and to improve our knowledge on the molecular basis and clinical-laboratory picture of FPD/AML.

Methods: Whole exome sequencing (WES) was performed in 86 propositi with an unknown IT after the diagnostic workup based on the most updated diagnostic algorithm for ITs (Clin Genet 2016;89:141). RUNX1 variants detected by WES were confirmed by Sanger sequencing in the propositi and all available family members, which also undergo clinical-laboratory characterization. The study was approved by the Institutional Review Board of the IRCCS Policlinico S. Matteo Foundation; all patients gave written informed consent.

Results: We identified three pedigrees (families 1-3) with different RUNX1 heterogeneous mutations, all segregating with thrombocytopenia in the respective families: the novel variants c.578T>A and c.967+2_5del, and the known c.351+1G>A. The thirteen individuals carrying the RUNX1 mutations had mild thrombocytopenia (platelet count ranging from 70 to 130 × 10^9/L) with mild bleeding tendency. Platelet sizes were within the normal range in all the six patients analyzed, and the serum level of thrombopoietin was normal or moderately increased. No specific morphological alteration of platelets was detected, except for moderate reduction in the alpha-granule content in family 1, confirmed by immunofluorescence analysis. The surface expression of the major platelet glycoprotein (GP) complexes GPIb-IIIa and GPIb-IX-V was normal. In family 1, a moderate reduction of GPIIa was detected, regardless of genotypes at the ITGA2 locus. A defective aggregation was detected after platelet stimulation with collagen 4 mcg/mL and ADP 2 mcM in the five patients investigated; normal responses were obtained using collagen 20 mcg/mL, ADP 20 mcM and ristocetin 1.5 mg/mL, suggesting mild functional platelets defects. Of note, the platelets from two families developed AML, with a prevalence lower than reported in literature, probably because of a different criteria of enrolment (RUNX1 germline mutations are usually searched in ITs associated with AML). No solid/hematological cancer was reported in family 1.

Summary/Conclusions: FPD/AML is an IT lacking pathognomonic laboratory criteria: it is characterized by a mild functional defect and, much more importantly, by a normal platelet size, similarly to the other ITs predisposing to hematological malignancies (ANKRD26 and ETV6-related thrombocytopenias). Given the importance of recognizing these diseases for patients counseling, follow-up, and therapeutic approach, we recommend a systematic screening for RUNX1, ANKRD26, and ETV6 mutations in all patients with an autosomal dominant IT and normal platelet size.

P366
Abstract withdrawn.

P367
A SINGLE-ARM, OPEN-LABLE, LONG-TERM EFFICACY AND SAFETY STUDY OF SUBCUTANEOUS ROMIPLOSTIM IN CHILDREN WITH IMMUNE THROMBOCYTOPENIA
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Background: The use of romiplostim in children with ITP has been evaluated in phase 1/2 and 3 studies. Here we describe children with ITP who will receive open-label SC romiplostim for up to 3 years (y).

Aims: To assess platelet responses in children with ITP receiving romiplostim.

Methods: Eligible children, recruited in 16 countries worldwide, had ITP for ≥6 months, ≥1 prior ITP therapy, and platelet (plt) counts ≤30×10^9/L. Weekly SC dosing started at 1 μg/kg and was titrated in 1 μg/kg increments up to 10 μg/kg to achieve plt counts of 50-200×10^9/L. The primary endpoint was the % of time in the first 6 months with a plt response (plt count ≥50×10^9/L).

Results: As of 15 Mar 2016, 145 patients received ≥1 dose. At baseline, median (min-max) age was 10 (2-17) y; 51% were female; 4% had prior splenectomy. Median (min-max) ITP duration was 1.9 (0.5-12.3) y and plt count was 13 (2-168)×10^9/L. The median (Q1, Q3)% of time with a plt response in the
first 6 months was 50% (0%, 83.3%); that of months 7-12 was 92% (33%, 100%). Overall, 80% (114/143) of patients had a platelet response. The median (Q1, Q3) of time with an increase in plt counts ≥20×10^9/L above baseline was 60% (25%, 84%). The median dose increased to 10 µg/kg by week 32. Median (min-max) treatment duration to day 25 was 21 (6-77) weeks for a total exposure to date of 79 patient-years. Median (min-max) average weekly thrombocytopenia decreases over the course of the study was 6.1 (0.4-9.0) µg/kg. 32 patients (22%) discontinued treatment for lack of efficacy (n=17), required other therapy (n=5), patient request (n=4), noncompliance (n=2), adverse event (AE) (n=2) (interstitial lung disease in a 15 y old boy and abdominal pain, vomiting, and headache related to treatment in a 9 y old girl), administrative decision (n=1), and investigator decision (n=1). 34 (23%) patients received rescue medications. 15 (10.3%) patients had serious AEs (SAEs) including epistaxis (n=4), petchiea (n=2), decreased plt count (n=2), and thrombocytopenia (n=2). A case of abdominal pain was the only SAE deemed treatment-related by the investigator. CTCAE grade 3 bleeding was seen in 8 patients (6%) and included epistaxis (n=5), ecchymosis (n=2), petchiea (n=2), and 1 case each of hematemesis, hemotoma, SC hemorrhage, injection site hemorrhage, and mouth hemorrhage. No grade 4 or 5 bleeding was observed. No neutralizing antibodies against rosimplost or TPO were identified. Of 30 patients with baseline bone marrow biopsies (bone marrow biopsies were obtained at European sites), all had modified Bauermeister scores of grade 0 (no reticulin) or 1 (fine fibers) and bone marrows typical for ITP. Of these 30 patients, 21 had evaluable on-study biopsies obtained after –1 year of treatment, with no increases in 2 or more grades, findings of collagen, or bone marrow abnormalities (Figure 1).

Summary/Conclusions: In this year 1 datacut of an ongoing open-label study of romplitost in children with ITP, the% of time in the first 6 months with a platelet response was 50%, with 80% of children having a platelet response at some point on study. The median rosimplost dose reached 10 µg/kg and there were no new safety signals. No effects of rosimplost were observed on the bone marrow in the subset of patients with bone marrow biopsies. Future datacuts for years 2 and 3 in this study, the largest of rosimplost in children with ITP with 97 patient-years of exposure to date, will provide more information on platelet response, dose requirements, and safety.

P368

NOVEL THIENOPYRIDINES AS POTENT PLATELET INHIBITORS: FUTURE TREATMENTS FOR PLATELET HYPERACTIVITY DISORDERS? N. Binsaleh1, C. Wigley1, K. Whitehead1, D. Moreno-Martinez1, S. Daniels1, S. Jones1, M. van Rensburg2, L. Pilkington2, D. Barker2, N. Dempsey-Hibbert1 1School of Healthcare Science, Manchester Metropolitan University, Manchester, United Kingdom, 2School of Chemical Sciences, University of Auckland, Auckland, New Zealand

Background: Platelet hyperactivity is associated with a number of disorders including Acute Coronary Syndromes (ACS) and manifests as increased platelet activation and often inappropriate thrombus formation. The thiopyridine class of anti-platelet drugs, of which clopidogrel and prasugrel are the most well known, target the P2Y12 receptor on platelets, blocking the effects of the platelet agonist ADP. However, the effect of these drugs is variable amongst patients, with some patients responding well and some remaining at risk of thrombosis. This variability highlights a need for a refinement of this class of P2Y12 inhibitor. The aim of this study was to assess the efficacy of six novel thiopyridine derivatives synthesized by our group by examining their potential as in vitro inhibitors of platelet function.

Methods: Healthy human platelets were isolated and incubated with novel thiopyridine compounds (DJ0081, DJ0199, DJ0201, DJ206, DJ0171, DJ0997) (10µM, 30min) prior to stimulation with ADP (10µM) and analysis of alpha granule secretion (CD62P expression), GPIIbIIa activation (PAKC-1 expression) and platelet leukocyte aggregate (PLA) formation using flow cytometry. Furthermore, light transmission aggregometry (LTA) was used to assess ADP-induced platelet aggregation after these treatments. As clopidogrel is usually prescribed in combination with the COX-1 inhibitor acetylsalicylic acid (ASA), synergy of the novel compounds with ASA (30µM) was also analysed by LTA. All results were compared to ADP-stimulated samples and samples treated with clopidogrel (10µM, 30min) prior to ADP stimulation.

Results: All six novel compounds demonstrated a significant reduction in ADP-mediated platelet aggregation (P <0.001), CD62P expression (p <0.001), PAC1 expression (p<0.01) and PLA formation (p<0.05). These compounds were also shown to enhance the inhibitory effects of ASA. DJ0171 and DJ0199 were particularly potent, displaying greater inhibitory effect than clopidogrel.

Summary/Conclusions: The study demonstrates the potential for new thiopyridine compounds as modulators of platelet function and points to the possibility of future use in patients at risk of platelet hyperactivity and thrombosis.

Figure 1.
Summary/Conclusions: In this cohort of patients from the International PNH Registry, treatment with eculizumab was associated with clinically meaningful improvements in PROs, including assessments of fatigue, global health status, patient functioning, and disease-related symptoms, as well as a decrease in emergency room visits and number of missed work days.

P370

ECONOMIC IMPACT OF INTRODUCING AGE-ADJUSTED D-DIMER CUT-OFF LEVELS IN THE DIAGNOSIS STRATEGY OF VENOUS THROMBOEMBOLISM

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Background: The diagnosis of venous thromboembolism (VTE) can be safely excluded in the case of D-dimer levels below a well defined cut-off value in patients with a low or intermediate pre-test probability (PTP) (as the test negative predictive value (NPV) is close to 100%). As age is associated with increased D-dimer levels, the question arose whether D-dimer measurement was useful to rule out VTE in elderly patients.

Aims: The aim of the present study was to evaluate the clinical performance of a diagnosis strategy based on age-adjusted cut-off values calculated by multiplying the patient’s age by 10 in patients aged over 50, and to evaluate its economic impact.

Methods: We included 1255 consecutive outpatients with non-high PTP of VTE referred to the emergency departments at 5 French centres (2 university hospitals, and 3 general hospitals, in whom D-dimer testing was prescribed. The same standardized procedure was used in the 5 centres i.e. D-dimer measurement in patients with a non-high PTP, and imaging techniques (usually computed pulmonary angiography in case of suspected PE and Doppler ultrasonography in case of suspected DVT) in the case of D-dimer above the cut-off level. D-dimer levels were evaluated using the same fully automated latex-based assay (HemosIL D-dimer, Instrumentation Laboratory), the usual cut-off level for VTE exclusion being 500 ng/mL (fibrinogen equivalent units, FEU).

Results: VTE diagnosis was established by objective testing in 115 patients (9.2%): 88 of the 1082 patients referred for suspected PE (8.1%) and 27 of the 173 patients referred for suspected DVT (15.6%). D-dimer levels were above 500 ng/mL in all patients with VTE and in 521 of the 1140 patients without VTE (45.7%), leading to test NPV and sensitivity of 100%. The overall test specificity was 54.3%, even though it significantly decreased in an age-dependent manner over 60 years old. This is due to increased D-dimer levels in older patients particularly in those above 80 years. Using age-adjusted cut-off levels, calculated by multiplying the patient’s age by 10, significantly improved the overall test specificity (60.2%). The NPV remained high (99.9%), even though a 78 y-old female with a low PTP of PE would have been misdiagnosed as her D-dimer level (540 ng/mL) was above 500 ng/mL but below the age-adjusted cut-off value. Such an improvement in test performance was found both in patients with suspected PE and DVT (Table). As such an increase in test specificity would have led to exclude VTE in a higher percentage of patients in the studied population, we evaluated the cost-effectiveness of both strategies, taking into account the local reimbursement rates of D-dimer testing, angiography and Doppler ultrasonography in (16.20, 58.72 and 75.60 Euros respectively). The economic impact of the proposed diagnosis strategy was a decrease of 6.9% versus the usual cut-off level (540 ng/mL) in all patients with VTE and in 521 of the 1140 patients without VTE (502.34 vs 48.356.4 Euros) for PE diagnosis and 5.1% (9.09 vs 10,438.2 Euros) for DVT. If such an analysis was used in the US, where angiography and Doppler US were more expensive (648 and 226 US$ respectively), and D-dimer less costly (14 US$), the cost savings would have been even higher (11.0% for PE, and -6.3% for DVT).

Summary/Conclusions: The use of age-adjusted cut-off levels for D-dimer, in patients aged over 50 years old, led to a significant increase in the test specificity, but corollatively to slightly decreased NPV and sensitivity. Even though some patients with D-dimer levels above 500 ng/mL but below the age-adjusted cut-off could be misdiagnosed, such a strategy was found to be safe in our studied population with a high NPV (99.9%) and sensitivity (99.1%), and cost-effective.

P371

IMPACT OF CELLULAR THERAPY ON THE ECONOMIC BURDEN AND SURVIVAL OF RELAPSE FOLLOWING AHSCT IN PATIENTS WITH ACUTE LEUKEMIA OR MYELODYSPLASIA

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Background: Relapse following allogeneic hematopoietic stem cell transplant (aHSCT) is associated to a very poor outcome and remains an unmet medical needs. The impact of treatment approach on costs and survival remains unknown. The development of innovative cellular therapy for the treatment of relapse following aHSCT may change its dismal outcome but the cost of such intervention has prohibited its large-scale development.

Aims: The objective of this study was to measure the economic burden associated with the management of relapse following aHSCT and to evaluate the impact of treatment choice on survival and health care costs.

Methods: A retrospective medical chart review was conducted at Maisonneuve-Rosemont Hospital (HMR) after research and ethic committee approval. Patients were selected using the Hematopoietic Stem Cell Transplant (HSCT) program database. Eligible patients were diagnosed with acute leukemia (AL) or MDS and relapsed following an aHSCT between January 1st 2011 and December 31st 2014. Patients’ and disease characteristics and relapse-related health care resource utilization were collected from the date of post transplant relapse until death or last follow-up. Canadian unit costs for each intervention/treatment were obtained from literature and governmental publications.

Results: During the study period, 645 HSCT were performed at HMR, 303 were allologenic. A total of 36 patients met the inclusion criteria and were included in the analysis. 32 recipients were diagnosed with AL and 4 with MDS. Treatment approaches following aHSCT relapse were divided in three groups according to patient and physician choices: group 1 received supportive care (n=9), group 2 received chemotherapy or tyrosine kinase inhibitors (n=21) and group 3 received a cellular based therapy, either donor lymphocyte infusion (DLI) or a second aHSCT (n=6). The mean cost of care per patient per month was C$20,239 (SD=17,079). The median survival following relapse for the entire cohort was 12.4 months (SD=2.8). For group 1, the mean cost of care per patient per month was C$17,436 (SD=16,447), C$22,914 (SD=18,474) and C$15,082 (SD=12,954), respectively. The median survival was 4.0 months (SD=2.0), 7.2 months (SD=1.6), and 46.6 months (SD=8.4), for treatment group 1, 2 and 3 respectively (Figure1).

Figure 1. Survival according to treatment group.

Summary/Conclusions: Relapse following AH SCT is associated to a poor prognosis and survival and to significant use of health care resources. Despite the selection bias, only patients who received cellular based therapy, either DLI or another HSCT, enjoyed a prolonged survival. Healthcare resources devoted to the care of patients in relapse post AHSCT provide a comparative basis for cost efficiency analysis in the development of innovative cellular therapy.

P372

ACUTE MYELOID LEUKEMIA TREATMENT PRACTICE PATTERNS, HEALTHCARE RESOURCE UTILIZATION (HRU) AND COSTS IN A US COMMERCIALLY-INSURED POPULATION

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Background: AML is a rapidly progressive hematologic malignancy that accounts for 25% of all leukemias in the Western World, with an estimated 5-year survival of 26%, and is associated with high HRU and costs.

Aims: To estimate HRU and costs among newly-diagnosed AML patients (pts) in a US commercially insured population by receipt of chemotherapy (CT) or stem cell transplant (SCT).

Methods: This was a retrospective observational study using the PharMetrics Plus® database. Pts were adults with AML (ICD-9-CM code 205.0x and corresponding ICD-10-CM codes) diagnosed between Jan 2007 and Jun 2016 (study period). Pts were excluded if: 1st AML claim was for remission/relapse;
not continuously enrolled for 12-months (mos) before the first AML claim (index date); evidence of acute promyelocytic leukemia anytime during the study period; missing enrollment information; or ≥1 hospitalizations during follow-up (FU) with missing cost. TPs were classified as treated or untreated, with treatment defined based on receipt of CT (inpatient or outpatient) or SCT. For treated pts, FU was partitioned into 2 periods: index date to 6 mos and >6 mos post index date. Mean HRU and costs over the FU period were calculated by receipt of treatment and, for treated pts, by time since index date.

**Results:** 10,197 pts met study criteria including 8,682 treated pts (67%) and 3,335 untreated pts (33%). Mean age was 55 and 60 years in treated and untreated pts, respectively. Mean follow-up was 19.3 mos in treated pts and 18.1 mos in untreated pts. Mean total costs were higher for treated pts ($386,711 vs untreated pts ($833,274). In treated pts, mean total costs were $166,156 during the first 6 mos (mean duration 3.9 mos), and $220,555 during the remaining follow-up period (mean duration 19 mos), 26% of treated pts had SCT. Costs of inpatient and outpatient CT during the first 6 mos were $86,188, representing 22% of the total cost for treated pts (Table 1).

### Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Treated Pts</th>
<th>Uncovered Pts</th>
</tr>
</thead>
<tbody>
<tr>
<td>S/C: RBC transfusions / platelets transfusions (final 3 mos)</td>
<td>$20.3/17.4 vs 13.7/17.5</td>
<td>$33.8/28.1 vs 48.5/29.5</td>
</tr>
<tr>
<td>Other vs treated vital signs (mean)</td>
<td>8.8/4.5 vs 25.8/7.5</td>
<td>22.8/40.1/25.9/31.4</td>
</tr>
<tr>
<td>Discharge to non-AL-PN</td>
<td>60.4/60.4</td>
<td>60.4/60.4</td>
</tr>
<tr>
<td>Discharge to non-AL-PN</td>
<td>60.4/60.4</td>
<td>60.4/60.4</td>
</tr>
<tr>
<td>Emergency department visits, mean</td>
<td>1.7</td>
<td>1.0</td>
</tr>
<tr>
<td>Inpatient, mean</td>
<td>3.9 vs 3.9</td>
<td>2.1 vs 2.1</td>
</tr>
<tr>
<td>Total costs, $0</td>
<td>198,321 vs 198,321</td>
<td>237,974 vs 237,974</td>
</tr>
<tr>
<td>SCT</td>
<td>$54,326 vs 54,326</td>
<td>$57,349 vs 57,349</td>
</tr>
<tr>
<td>Other</td>
<td>$10,339 vs 10,339</td>
<td>$12,148 vs 12,148</td>
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<tr>
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<td>$12,148 vs 12,148</td>
<td>$14,958 vs 14,958</td>
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<tr>
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<td>$17,768 vs 17,768</td>
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<tr>
<td>Total</td>
<td>$200,208 vs 200,208</td>
<td>$254,084 vs 254,084</td>
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</table>

**Summary/Conclusions:** HRU and costs of managing AML pts are considerable, with greatest HRU and cost in pts receiving CT or SCT.

### P373

**HEALTH-RELATED QUALITY OF LIFE IN AL AMYLOIDOSIS PATIENTS WITH NERVOUS SYSTEM INVOLVEMENT**

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**Background:** In light chain (AL) amyloidosis, misfolded light chains accumulate and cause progressive peripheral neuropathy (PN) and failure of critical organs such as the heart and kidneys. Consequently, a progressive, ascending seniormotor neuropathy is often a related clinical finding.

**Aims:** This study describes disease characteristics and health-related quality of life (HRQoL) in AL amyloidosis patients with peripheral nerve involvement (AL-PN).

**Methods:** An online survey was administered to AL-PN (n=126) and non-nerve–affected (n=215) patients to assess patient characteristics and HRQoL (based on the SF-36v2 Health Survey [SF-36v2]). The survey measures eight domains: physical function, role physical (PF), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role emotional (RE), mental health (MH), in addition to physical (PCS) and mental component summary (MCS) measures. Patient characteristics were compared using chi-square tests. Differences in symptomatic and HRQoL burden were tested with multivariable logistic and linear models, respectively. Differences in mean HRQoL between AL-PN and non-AL-PN patients were compared to established minimally important differences (MIDs).

**Results:** Compared to non-nerve–affected patients, greater proportions of AL-PN patients visited ≥6 doctors (42.1% vs 19.5%, p <0.001) and ≥3 specialists (24.6 vs 9.9%, p <0.001). AL-PN patients also had symptoms for ≥1 year prior to receiving a diagnosis (50.8 vs 39.1%, p=0.035), relative to non-nerve–affected patients. Nearly all AL-PN patients (97.6%) reported multi-system involvement. Gastrointestinal involvement was more prevalent in AL-PN patients as compared to non-AL-PN patients (p<0.05 for all). These significant differences also exceeded the thresholds for clinically meaningful differences between the two groups.

**Summary/Conclusions:** This study suggests that the burden of illness from AL amyloidosis may be greater for those with PN involvement versus those without. AL-PN patients also experienced more complicated journeys to diagnosis and significantly worse symptoms related to nervous systems and physical HRQoL. The SF36v2, a reliable and valid assessment of HRQoL in AL amyloidosis studies, was sensitive to differences in HRQoL between AL-PN and non-AL-PN patients. Future research should examine whether improvements in neuropathy symptoms following treatment subsequently lead to improvements in HRQoL among patients with AL-PN. These findings are helpful for patient-focused drug development and supportive treatments.

### P374

**ACCESS TO COMMUNITY CHEMOTHERAPY IMPROVES PATIENT QUALITY OF LIFE**

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**Background:** Deciding how severe a disease is for people with haematological cancers are to be delivered is going to be an important challenge in the coming years. Clinics have limited clinic capacity in terms of staff and bed space to cope with increased demand. In Wales many day units are already at capacity, overcrowded and have long waits for treatment. Ambulatory care, including diagnos-osis, observation, consultation, intervention, and rehabilitation, has the potential to improve patient experience, if traditionally-based hospital services are moved into the community. We used a Mobile Unit – a 34-tonne articulated lorry which opens out to become a bespoke clinical space - to deliver treatments in a community setting to a range of haematology patients for a period of 12 months in South Wales.

**Aims:** We aimed to explore whether the administration of cytotoxic therapy on a Mobile Unit in a community setting for patients with haematological cancers could prove to be a safe and efficient alternative to hospital therapy, and in particular whether this model of service delivery would be acceptable to patients. Our target group was patients with myeloma, aiming for up to 20 a day once or twice a week.

**Methods:** The first drugs administered on the Mobile Unit were zoledronate infusions, followed by bortezomib. When twice weekly doses were required, patients collected an additional injection pack which they could self-administer in their own homes, thereby saving another trip to hospital. Immunoglobulin infusions, taking between 1-2 hours, were also administered. There was a con- sultant review clinic on board for patients receiving bortezomib which further reduced the numbers of hospital visits for patients and also a nurse-led Quality of Life (QoL) clinic.

**Results:** In one year 548 treatments were administered on 91 days to a total of 54 individual patients. All 54 patients had a diagnosis of myeloma. 56% were female and 44% are male with an age range of 46 to 90 years of age, with 48% over 70 years of age. 37 patients are married and all but 4 classified themselves as White British. The greatest number of patients treated in a single day was 16. 98% felt safe having their treatment outside hospital and 92% said their experience was better than hospital. Patients could drive right up to the door of the Mobile Unit and average time waiting from arrival to treatment chair was 2 minutes, with many patients not having to wait at all. Uptake of the psychosocial support services was lower than expected with only 10 people opting for additional support. Any criticisms received focused on the locations we chose to site the Mobile Unit in relation to accessibility via public transport.

**Summary/Conclusions:** Treatment in the community alleviates the stress of treatment and with minimal waiting times it gives some patients the ability to maintain family life and where possible to continue to work. It is both feasible and acceptable to begin to ambulate many different sorts of treatments. The possibilities opening up for haematology include rituximab maintenance; community blood transfusions; delivering pentamidine for patients at risk of pneumocystis infection; late effects clinics for teenage and young adult cancer patients; and myeloproliferative neoplasm clinics, possibly near community pharmacies to facilitate dispensing medicines such as hydroxyurea.
which will have a substantial price difference compared with nilotinib. However, given the possible changes in switching of TFR, this price difference may not translate into a similar magnitude of difference in drug budget for first-line nilotinib vs imatinib due to better MR with nilotinib.

Aims: To estimate the budget impact for first-line nilotinib vs imatinib when considering generic imatinib pricing, early treatment-switching, and TFR.

Methods: The model was based on ENESTnd and ENESTfreedom trial data and TFR use on clinical outcomes and treatment costs. Analyses were run for 1000 patients with newly diagnosed CML, starting either nilotinib or imatinib, over a 5-year time horizon and using French drug pricing. It was assumed that all patients in the model would switch therapy (imatinib to nilotinib, and nilotinib to dasatinib) based on the failure criteria of the ELN guidelines. As such, ENESTnd trial data were re-analyzed to estimate switching based on the model. The model assumed that patients could enter first-line or second-line TFR after 36 months of continuous therapy where the last 12 months were at MR4.5. Duration of first-line or second-line TFR was based on an extrapolation of ENESTnd trial data for treatment-free and survival curves, respectively. Monthly drug costs were €2,952 for first-line nilotinib and €1,063 for generic imatinib, assuming a 50% discount to brand pricing.

Results: A greater number of patients in the first-line nilotinib arm remained on first-line therapy (690 vs 479 at 15 mos., and 542 vs 366 at 60 mos.); achieved MR4.5 on first-line therapy (347 vs 183 by 60 mos.); entered TFR on first-line therapy (347 vs 183 by 60 mos.); and entered TFR on either first- or second-line (494 vs 400 by 60 mos.); and was in any TFR at 60 months (293 vs 200). The incremental budget impact per patient for first-line nilotinib vs imatinib decreased each year from €16,482 in Year 1 to €377 in Year 5. Overall, the 64% lower drug acquisition costs per patient of imatinib (€1,063) vs nilotinib (€2,952) provided only a 17% lower total budget impact over five years (€141,204 vs €170,002) per patient.

Summary/Conclusions: Results from the model considered more switching as per 2013 ELN guidelines, which resulted in greater and quicker switching than currently described in ENESTnd. Overall, it was projected that patients on imatinib, patients who receive first-line nilotinib would have earlier and more sustained molecular response requirements for TFR eligibility—and be subject to less treatment-switching. The model projected that less than 50% of patients would remain on first-line imatinib at 15 months. This would significantly reduce the budget benefit of a lower imatinib acquisition price. The potential impact between first-line imatinib and nilotinib would be further reduced by TFR, which occurred in the model more frequently in the nilotinib group. The superior efficacy of nilotinib and the associated differences in switching and TFR eligibility are predicted to substantially offset the lower unit cost for generic imatinib.

P376
GAH SCALE PREDICTS TREATMENT TOLERABILITY IN OLDER PATIENTS (>65 YEARS) DIAGNOSED WITH HEMATOLOGICAL MALIGNANCIES (Abstract P376)

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Background: The Geriatric Assessment in Hematology (GAH) scale is a newly developed tool that is intended to be an ancillary questionnaire to better categorize patients diagnosed with hematologic disorders for intensive treatment in routine clinical practice. It is a brief (<12 min) and easy to administer instrument, which takes into account 8 dimensions of geriatric assessment that were initially dichotomized into 0 or 1. The GAH scale has recently been shown to be psychometrically valid, responsive to clinical change, and able to predict survival.

Aims: To determine the weights for each dimension of the GAH scale and the cut-off points for the scale to be used as a tool to predict treatment tolerability in older patients diagnosed with myelodysplastic syndrome / acute myeloblastic leukemia, multiple myeloma, or chronic lymphocytic leukemia.

Methods: A retrospective, observational study conducted at 14 Spanish sites. Prior participants of the GAH study were given treatment within 3 months after having completed the GAH scale were eligible for inclusion after giving informed consent.

A logistic regression model and a full multiple linear regression model were calculated to determine the weights for each dimension and its contribution to the final score; the ROC curve analysis was used to calculate the cut-off points that defined three groups: “go-on” (low probability to develop toxicity regardless of intensive or attenuated therapy), “slow-go” (high probability to develop toxicity with intensive therapy but low probability with attenuated therapy), and “low-go” (high probability to develop toxicity with first-line therapy). The coefficients of the dimensions are > 7 for number of drugs, -10 for gait speed, 2 for mood and nutritional deficiencies (e.g., hypocholesterolemia, hypoalbuminemia, weight loss). Diets rich in fruits, vegetables, legumes, whole grains, fish, nuts, and low-fat dairy products are associated with a decrease in inflammatory (e.g., TNF-a, IL-6, and CRP) and thrombogenic markers (e.g., homocysteine, fibrinogen; Chrysohoou 2004, Smidowicz 2015). To date, no studies have evaluated the nutritional needs or preferences of MPN patients in regards to dietary change.

Aims: The aim of this project was to determine the nutritional needs and preferences that will help inform the creation of a tailored MPN dietary intervention.

Methods: An internet-based survey was hosted by the Mayo Clinic Survey Center and promoted on multiple Mayo Clinic social media pages and websites during February of 2017. The survey included data on demographics, MPN characteristics, nutritional habits, supplement use, and symptom burden using the MPN-SAF TSS/MPN-10 (Emanuel 2012).

Background: Cachexia, weight loss, and malnutrition in cancer patients are important contributors of adverse outcomes of cancer patients. MPN patients have abnormal cytokine expression (e.g., IL-1, IL-6, IL-8, and TNF-a) that contributes to symptom burden (e.g., fatigue, pruritus, night sweats, bone pain) and a cytokine deficiency (e.g., MPN-SAF TSS/MPN-10) which contributes to symptom burden (e.g., fatigue, pruritus, night sweats, bone pain) and a cytokine deficiency (e.g., TNF-a, IL-6, CRP) and thrombogenic markers (e.g., homocysteine, fibrinogen; Chrysohoou 2004, Smidowicz 2015). To date, no studies have evaluated the nutritional needs or preferences of MPN patients in regards to dietary change.

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Results: Demographics and symptom burden: 919 international MPN patients (187 MPN-SAF TSS ≥ 40, 896 MPN-SAF TSS ≥ 40) were enrolled in the survey. Overall, 34.4% of patients endorsed a greater number of patients in the first-line nilotinib arm remained on first-line therapy (690 vs 479 at 15 mos., and 542 vs 366 at 60 mos.); achieved MR4.5 on first-line therapy (347 vs 183 by 60 mos.); entered TFR on first-line therapy (347 vs 183 by 60 mos.); and entered TFR on either first- or second-line (494 vs 400 by 60 mos.); and was in any TFR at 60 months (293 vs 200). The incremental budget impact per patient for first-line nilotinib vs imatinib decreased each year from €16,482 in Year 1 to €377 in Year 5. Overall, the 64% lower drug acquisition costs per patient of imatinib (€1,063) vs nilotinib (€2,952) provided only a 17% lower total budget impact over five years (€141,204 vs €170,002) per patient.

Summary/Conclusions: Results from the model considered more switching as per 2013 ELN guidelines, which resulted in greater and quicker switching than currently described in ENESTnd. Overall, it was projected that patients on imatinib, patients who receive first-line nilotinib would have earlier and more sustained molecular response requirements for TFR eligibility—and be subject to less treatment-switching. The model projected that less than 50% of patients would remain on first-line imatinib at 15 months. This would significantly reduce the budget benefit of a lower imatinib acquisition price. The potential impact between first-line imatinib and nilotinib would be further reduced by TFR, which occurred in the model more frequently in the nilotinib group. The superior efficacy of nilotinib and the associated differences in switching and TFR eligibility are predicted to substantially offset the lower unit cost for generic imatinib.
using diet to help control their symptoms or MPN disease. Patients most often utilized books (28.2%), websites (27.1%), health care providers such as physicians, NPs or naturopaths (28.2%), online forums (23.2%), friends (12.2%), nutritionists (9.5%), phone or tablet applications (9.1%), or videos (4.2%) for nutritional education. The vast majority (95.9%) of MPN patients endorsed being willing to eat only certain foods if it helped to control symptom burden and or could help their MPN to stabilize or reduce the risk of their MPN getting worse (98.0%).

Table 1.

Summary/Conclusions: There remains an unmet need for symptom burden improvement in low-risk MPN patients or among those who have reoccurrence of symptoms while on JAK inhibitor therapy. Nutritional interventions for MPN patients have not previously been investigated and have the potential to be paired with traditional interventions to allow MPN patients to self-manage symptom burden. This study represents the first evaluation of MPN-related nutritional habits and preferences. These results will be used to inform the creation of an MPN nutritional intervention with the goal of improving symptom burden and reducing inflammation.

Summary/Conclusions: DO PHYSICIANS NEED HELP TO ADEQUATELY INFORM AND SUPPORT PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA? RESULTS FROM A QUALITATIVE STUDY IN GREECE

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Background: Despite recent progress in prognostication and management, chronic lymphocytic leukemia (CLL) remains unpredictable at diagnosis, while virtually incurable, posing challenges to physicians on how to properly communicate the actual nature of the disease. Moreover, the great majority (~85%) of patients do not need treatment at diagnosis, creating a major cognitive dissonance between the perception of leukemia diagnosis and the "wait & watch" strategy usually applied, that may become a major reason of anxiety and quality of life (QoL) impairment for patients and frustration for physicians. Evidently, both patients and physicians need parameters that would allow co-decision making tailored to each particular case.

Aims: To identify physicians' needs in order to improve their communication skills and thus facilitate CLL patient empowerment through a patient-centered-ness model.

Methods: An in-depth qualitative study with semi-structured interviews was conducted within hematologists (n=30) all over Greece. Data collection was considered as completed when saturation was reached i.e. no new themes emerged as assessed by the investigators. Content analysis was performed separately by a hematologist and a health psychologist with 98% inter-rater reliability score.

Results: None of the participants had ever received formal communication training but rather adopted the techniques of senior physicians or developed their own through experience alone, thus frequently doubting their approaches (n=12/30, 40%). The most popular communication technique mentioned was adaptation of the quality and quantity of information provided according to each patient's characteristics (n=29/30, 96.7%); followed by the use of caregivers as mediators for the communication of difficult issues (n=24/30, 80%); balance of realism and hope (n=21/30, 70%); careful choice of wording (e.g. lymphocytosis instead of leukemia) (n=18/30, 60%); gradual disclosure (n=17/30, 56.7%); and, descriptions through pictorial representations or metaphors (n=16/30, 53.3%).

Even though physicians did not systematically assess patients' anxiety and depression levels, they often found themselves dealing with patients' emotions (n=29/30, 96.7%) through lengthy discussions. With regards to decision making, some mentioned that physicians should make all the decisions (n=9/30, 30%) and that patients are not always willing to take part in the decision-making process (n=8/30, 26.7%), while others were keener on stirring patients towards a decision (n=15/30, 50%); taking into account patients' preferences (n=10/30, 33.3%). Most physicians felt uncomfortable delivering bad news such as initial diagnosis, relapse and poor prognosis (n=25/30, 83.3%). Self-reported needs included (i) communication skills training (n=20/30, 66.7%); (ii) psychological support (n=7/30, 23.3%); and, (iii) working in a multidisciplinary team (n=8/30, 26.7%).

Summary/Conclusions: In the absence of structured communication guidance there is great uncertainty among physicians concerning their skills on communicating CLL nature and handling difficult situations, leading to distress endangering their engagement in a healthy relationship with the patient. Additional studies are warranted at European level for identifying physician needs in different countries aiming at improving their communication skills to support and empower CLL patients for participating in their own care and enhance their QoL.
OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLAN-
TATION FOR PATIENTS WITH ACUTE LEUKEMIA ABOVE 70 YEARS OF
AGE: ON BEHALF OF THE ACUTE LEUKEMIA WORKING PARTY OF THE
EBMT

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ogy/Oncology, Medical Center Drive, Nashville, United States, 4Hospital Saint
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Background: The average age of patients (pts) with AML is about 67 years. Historically, many of these pts were not considered as viable candidates for allo-
geneic transplantation (HCT) because of concerns about increased transplanta-
tion-related toxicity and excessive non-relapse mortality (NRM), a challenging
problem especially in older individuals. However the development of reduced-
toxicity conditioning (RIC) regimens and the improvement in HCT supporting
care allowed the successful application of HCT in older pts with AML.

Aims: Compare outcome of allo SCT in acute myeloid leukemia AML patients
aged above 70 years of age with that of younger patients.

Methods: AML patients aged between 50 and 90 years old receiving a first or
second allo SCT between 2004 and 2014 with MSD or UD donor were included
in the study. Comparison of outcomes of patients aged above 70 with that of
patients between 50-70 years were performed for the whole group and sepa-
rate analyses according to disease status at SCT (CR1, CR2, above).

Results: Altogether N=16874 pts were included in the study, N=713 were aged
above 70 years old (median 72, IQR 71-73) and N=16161 between 50 and 70
(median 59, IQR 55-63). Older pts were more often male (62 vs 55%, p<0.001),
had more often secondary AML (42% vs 28%, p<0.001), more advanced dis-
ease (42% vs 27%, p<0.001), more often peripheral blood cell grafts (96
vs 91%, p<0.001), more often unrelated donors (79% vs 59%, p<0.001) and
poorer Karnofsky score (36% below 90, p<0.001), received more often reduced
intensity conditioning (80 vs 63%, p<0.001). Incidence of acute GVHD
II/III/IV, chronic GVHD and relapse were the same in the two groups in mul-
tivariate analyses. Non-relapse mortality (NRM) at two years was 34% (95%CI
31%-38%) in pts above and 24% (25%-32%) in those below 70 years of age
(p<0.001). Overall survival and leukemia-free survival (LFS) at 2 years was
38% (95%CI 34-42) vs 50% (95%CI 49-50) p<0.001 and 33% (95%CI 29-37)
vs 44% (95%CI 43-45) in the two groups, respectively (p<0.001). Among pts
in CR1, 2 years survival was 43% (95%CI 37-51) vs 57% (95%CI 56-58)
(p<0.001), in CR2 it was 36% (95%CI 27-27) vs 52% (95%CI 50-54) (p=0.002)
and in advanced disease 35% (95%CI 29-41) vs 33% (95%CI 31-34) (p=0.36)
in pts above and below 70 years of age, respectively. Among pts older than 70
years of age a Karnofsky score >80 was associated with improved survival
and LFS in multivariate analysis (HR 0.7 95%CI 0.5-0.9 , p=0.005 and HR 0.7
95%CI 0.5-0.9 , p=0.003 respectively).

Summary/Conclusions: In AML with CR1, CR2 status at allo SCT, pts above
70 years of age have worse NRM, survival and LFS compared to pts 50-70
years of age. In pts above 70 years of age Karnofsky score is of significant
importance for outcome.

OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLAN-
TATION FOR PATIENTS WITH ACUTE LEUKEMIA ABOVE 70 YEARS OF
AGE: ON BEHALF OF THE ACUTE LEUKEMIA WORKING PARTY OF THE
EBMT

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importance for outcome.
Sickle Cell Disease (SCD) and Diamond-Blackfan Anemia (DBA). Bertain et al (Blood, 2014) have previously shown that αβ TCR depleted haplo-transplantation in children with multiple types of non-malignant disorders was feasible. An ongoing Phase II/III trial evaluates the safety and efficacy of post-transplant infusion of donor T-cells transduced with the iC9 suicide gene (BPX-501 cells). The iC9 vector contains the sequence for the CD19 marker, so that the BPX-501 cells (CD3+/CD19+) can be tracked in peripheral blood. We report on 15 children with hemoglobinopathies and ED.

**Aims:** This study was performed to determine the clinical impact of infusion of BPX-501 T cells post αβ T-cell depleted haplo-identical HSCT in pediatric patients with hemoglobinopathies.

**Methods:** Fourteen patients were transplanted from a parent and one patient was transplanted from a sibling. Conditioning regimen included busulfan, thiotepa and fludarabine. Low dose ATG was administered to prevent graft-versus-host disease (GVHD) and graft failure. No post-transplantation GVHD prophylaxis was given. Median follow-up is 387 days (range 126-631 days).

Six patients were males and nine females, and median age at diagnosis and at HSCT was 0.8 and 8.9 years (range 2.5-19.2), respectively. Two patients had DBA and four with SCD. All 9 TM patients were βo/βo, and among the those with TM, 4 patients belonged to class I and 3 to class II of the Pesaro classification. All 15 patients were transfusion-dependent and receiving iron-chelation therapy before haplo-HSCT. 13/15 patients maintained full donor chimera. The patients with secondary graft failure were re-transplanted from the same donor and maintained full donor chimera.

**Results:** All patients are alive and well with no Treatment Related Mortality (TRM). Initial immune reconstitution on treatment 0 ( Range 0-6 ) and median time to platelet recovery was 11 days (range 8-12 days). Median time to last RBC transfusion was 8 days (5 – 34 days). See Figure 1 for individual Hemoglobin levels. Median time of infusion of 1x10^6 BPX-501 T cells/kg was 14 days after HSCT (range 10-26). BPX-501 cells expanded after infusion and still persist in all patients. Immune reconstitution was normal cellular and humoral immunity present at 186 days post HSCT. All patients remain transfusion-free with a median hemoglobin of 11 or greater after 6 months.

**Figure 1.**

**Summary/Conclusions:** These data suggest that Haplo-HSCT combined with infusion of BPX-501 T cells with a suicide gene may be a safe and curative option for children with hemoglobinopathies and ED who lack a matched donor. Infusion of gene modified T cells with an inducible suicide mechanism, combined with selective αβ T-cell depletion, offers the potential to rapidly reverse GVHD and eliminate the need for the use of GVHD prophylaxis. Additionally, this approach results in rapid hematopoietic and immune reconstitution for Haplo-HSCT recipients.

P382

EXCELLENT RESPONSE, LOW TRM AND GOOD SURVIVAL IN PATIENTS WITH THERAPY-REFRACTORY aGVHD AFTER TREATMENT WITH EQUIPOTENT MSCS FROM A SERUM-FREE MSC-BANK GENERATED FROM POOLED BM-INDUCED MULTIPLICITY-DERIVED CULTURES


**Aims:** This approach results in rapid hematological and immune reconstitution for HSCT recipients. In this approach results in rapid hematological and immune reconstitution for Haplo-GvHD and eliminate the need for the use of GvHD prophylaxis. Additionally, to all patients highly similar MSC products, we established a proprietary pooling procedure and generated a large bank of MSC end-of-passage-1 vials from which end-of-passage-2 MSC products are expanded for clinical use. The manufacturing process is fully GMP-compliant and generates an animal-serum-free product with novel anti-inflammatory and reduction of immune potency. Importantly, they showed a significantly higher allo-suppressive potential than the mean allo-suppressive potential of MSCs generated from individual donors. All tested individual MSC doses were equipotent in suppression of the allogeneic T-cell reaction in mixed lymphocyte reactions (Kuc1 et al. Haematologica 2016. 101 (8): 985-994).

**Methods:** Using these standardized MSC products altogether 52 patients were treated between December 2014 and December 2016. Patients were male (n=31, 60%) or female (n=21, 40%) and were transplanted for leukemia (n=38, 73%), or non-malignant (n=14, 27%) diseases. Median age was 8y (range: 0.5-52 years) and 5/8 cells were female (n=17%). 1% were MMFD and 1% were MMFD (n=10, 19%) and derived from BM (n=27, 52%), peripheral blood (n=24, 46%) or cord blood (n=1, 2%). Patients were suffering from aGVHD grade II (n=3, 5.5%), III (n=14, 27%), or IV (n=31, 60%) or extensive cGVHD (n=4, 7.5%). Acute GVHD occurred at a median of 52 days (5-280 days) after transplant. Patients received in weekly intervals up to four MSC infusions after having failed to respond to the treatment with either two lines (n=10, 19%), three lines (n=20, 38%), four lines (n=10, 19%), 5 lines (n=7, 13%), six lines (n=4, 8%), or 7 lines (n=1, 2%) of immune suppressive drugs.

**Results:** Response was defined as either complete response (CR) in patients who showed one overall GVHD grade less according to the Glucksberg criteria, or non response (NR) at day 28 after first MSC transfusion. At day +28, 12 patients (23%) achieved CR, 29 patients (57%) PR (overall response= 80%), 8 patients (17%) NR, and in 2 patients (4%) no data were available at day +28. At the last follow up of GVHD, 29 patients (56%) were in CR, 13 patients (25%) in PR, 9 patients (17%) in NR, and for 1 patient (2%) no data were available. At 2 years these response rates resulted in a non-relapse mortality rate (NRM) of 27±6%, cumulative relapse incidence (CIR) of 14±4%, and overall survival (OS) of 80±10%. Patients with aGVHD III and IV had an OS survival probability at 2 years of 77±12% and 59±35%, respectively thus dramatically in excess of expected survival rates for patients with such severe aGVHD. There was no difference between younger (n=40) and older patients (n=12) than 16 years.

**Summary/Conclusions:** Treatment with standardized equipotent MSCs from the FRANKFURT MSC-BANK offers an excellent chance to overcome treatment-resistant and steroid-refractory acute GVHD.

P383

HIGHER PEAK TACROLIMUS CONCENTRATIONS AFTER ALLOGENEIC TRANSPLANTATION INCREASE THE RISK OF ENDOTHelial CELL DAMAGE AND COMPLICATIONS

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**Aims:** “Hospital exemption” issued by the national regulatory authority Pau-Ehrich-Institute (Number: PEI. A.11748.01.1) licenses the clinical use of these products for patients with steroid refractory GVHD. On the basis of this licence patients were with severe GVHD treated who were either non responsive to the treatment with any other treatment or who were resistant steroids after 7 days.

**Methods:** Patients were male (n=31, 60%) or female (n=21, 40%), n=20 (40%) with severe aGVHD. There was no difference between younger (n=40) and older patients (n=12) than 16 years.

**Summary/Conclusions:** HIGHER PEAK TACROLIMUS CONCENTRATIONS AFTER ALLOGENEIC TRANSPLANTATION INCREASE THE RISK OF ENDOTHelial CELL DAMAGE AND COMPLICATIONS
and pts with unavailable TAC concentration data were excluded. A total of 253 pts was eligible. All pts received standard GVHD prophylaxis by continuous intravenous (iv) TAC with starting dose of 0.02 mg/kg/day from 1 day before allo-HSCT (day -1) and iv methotrexate on day 1, 3, 6 at dose of 10 mg/m2, 7mg/m2, respectively. TAC dosage was adjusted to target the serum concentration of 8-12 ng/ml until at least day 30 and then tapered. TAC was rapidly tapered in case of the pathological diagnosis of TAM. TAC serum concentration was sequentially examined tri weekly until day 35 at least. The primary endpoint of this study was to evaluate the cumulative incidence of TRC-EC in relation to weekly mean/peak TAC concentration. Secondary endpoint was OS.

**Results:** Median patient age was 45 years (16-68). The risks of disease were standard in 168 and high in 85 pts. Forty pts were diagnosed of TRC-EC: SOS: 7 pts (median onset: day 24 (17-40)), TAM: 27 pts (median onset: day 40 (25-128)), TTP: 6 pts (median onset: day 161.5 (46-233)). The cumulative incidence of TRC-EC at day 250 was 0.16 (95%CI, 0.12-0.21). Univariate analysis showed that higher peak TAC concentrations (PTC) during day 22-28 (P=0.013), male pts (P=0.04) and pts of reduced intensity conditioning regimen (P=0.01) were significantly associated with poor OS. In multivariate Fine-Gray analysis, high PTC during day 22-28 (HR: 1.92, 95%CI, 1.07-3.45, P=0.026) and grade 4 acute GVHD (HR: 8.33, 95%CI, 4.18-16.59, P<0.01) remained associated with TRC-EC occurrence. The probability of OS at 15-months was 0.56 (95%CI, 0.47-0.64). Univariate analysis showed that pts diagnosed TRC-EC (P=0.01), pts older than 50 (P<0.01), pts with high disease risk (P<0.01) and pts who received reduced intensity conditioning regimen (P=0.01) were significantly associated with poor OS. PTC and MTC at any time-point were not significant factors for OS. By Cox proportion-al-hazards regression models, TRC-EC diagnosis: (HR: 1.90, 95%CI, 1.16-3.11, P=0.011) and high disease risk at transplant: (HR: 1.76, 95%CI, 1.14-2.73, P=0.011) were significantly associated with poor OS (Figure 1).

**Summary/Conclusions:** Higher peak TAC concentrations during 22-28 days after allo-HSCT increased the risk of TRC-EC. And the development of TRC-EC was associated with poor OS.

**P384**

**IMPACT OF CONDITIONING REGIMEN ON OUTCOMES OF T-REPLETE HAPLOIDENTICAL TRANSPLANTATION FOR PATIENTS OVER 45 YEARS-OLD WITH AML: A STUDY ON BEHALF OF THE ACUTE LEUKEMIA WORKING PARTY OF THE EBMT**


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**Background:** T-cell replete haplo-identical stem cell transplantation (haploSCT) is a valid therapeutic option for adult patients (pts) with high risk acute myeloid leukemia (AML) lacking a sibling or unrelated donor. However the impact of reduced intensity (RIC) vs myeloablative (MAC) conditioning regi-

ons is not conclusive as no randomized study addressing this question is yet available.

**Aims:** In the present study we compared the outcome of RIC and MAC in pts with AML older than 45 yrs undergoing haploSCT. The aim of the study was to confirm the efficacy and feasibility of RIC among a population for which the choice of conditioning intensity is more related to center strategy than pts comorbidities or disease status.

**Methods:** We retrospectively compared the outcomes of 614 pts with de novo or secondary AML transplanted between 2007 and 2015 from an haplo-identical donor using either RIC (n=365) or MAC (n=249) regimens. Age was categorized in three subgroups (45-55 yrs, 55-60 yrs, >60 yrs). Patients receiving a previous allogeneic transplantation were excluded. RIC was defined according to EBMT definitions.

**Results:** The median follow up for MAC and RIC was 24 and 20 months, respectively and the median year of transplant was 2013 for both. Pts receiving a RIC were older (55 yrs in MAC vs 61 yrs in RIC, p<10^-4). Secondary AML was more frequent in RIC vs MAC (31% vs 22%) while 77% of MAC and 68% of RIC were transplant for de novo AML, p=0.01. No differences were found on disease status and Karnofsky performance status (KPS) at transplant: pts were in CR1 (MAC 44%, RIC: 40.5%), CR2/3 (MAC: 17%; RIC: 17%) or had active disease (MAC: 40%; RIC: 43%), p=0.68; 12% of pts in both groups had KPS>80, p=0.95. The most frequently used MAC regimen was TBI/Fludarabine (24%). RIC regimen was used more frequently by centers with associated hematopoietic stem cell source (MAC 42% vs RIC 55%, p=0.002). Post-transplant cyclophosphamide was used in 69% of both RIC and MAC, p=0.39. Main outcomes were not different according to conditioning regimen: at 2 years RI was 26% vs 32% (p=0.29), NRM 31% vs 34% (p=0.62), aGVHD grade II-IV 24% vs 31% (p=0.05), and cGVHD 27% vs 26% vs 39% vs 39% (p=0.17). OS 46% vs 39% (p=0.15), DFS 36% vs 28% (p=0.10) for MAC vs RIC, respectively. The results according to RIC and MAC were not different in any of the three age subgroups. 338 patients died; main causes of death were infections and GVHD to be fol-

lowed by disease recurrence. In multivariate analysis, the type of conditioning regimen was not associated with risk of relapse or treatment failure: RI (HR: 1.22, p=0.28), NRM (HR: 0.92, p=0.63), acute GVHD grade II-IV (HR: 1.14, p=0.48), chronic GVHD (HR: 1.26, p=0.30), LFS (HR: 1.03, p=0.77), GRFS (HR: 1.07, p=0.55), OS (HR: 1.05, p=0.68). Disease status was associated with outcomes (acute disease vs CR): RI (HR: 2.44, p=<10^-4), LFS (HR: 1.75, p<10^-4), GRFS (HR: 1.72, p<10^-4) as well as KPS>90: NRM (HR: 0.53, p=0.0002), LFS (HR: 0.67, p=0.001), GRFS (HR: 0.74, p=0.014), OS (HR: 0.62, p=0.0002).

**Summary/Conclusions:** In our study no differences were found between RIC and MAC regimens for haplo-SCT in adults with AML including the age stratified population. Disease status and performance status were the major predictors of transplantation outcome, while conditioning intensity had no effect. These results may serve as the background for a well design randomized study com-

paring RIC vs MAC for haplo-SCT in adult pts with AML.
Decision analysis is a computerized modeling analysis which can simulate the clinical outcomes of different therapeutic strategies and identify an appropriate therapeutic strategy.

Aims: The aim of this study is to compare the life expectancy (LE) of chemotherapy followed by up-front allo-HSCT to that of chemotherapy alone using decision analysis in patients with aggressive ATL using database constructed by a nationwide survey.

Methods: We constructed a Markov decision analysis model to compare the outcomes in 2 therapeutic strategies: chemotherapy followed by up-front allo-HSCT vs chemotherapy alone. The transition probabilities between each health states were calculated from the database of 1,792 patients and patients were stratified into low-, intermediate- and high-risk groups according to the risk stratification system which we developed previously (Fuji S et al. 18th International Conference on Human Retrovirology). The model simulated the LE, quality-adjusted LE (QALE) and survival curve after diagnosis of aggressive ATL. Since QoL data for patients with aggressive ATL are lacking, estimates for QoL were taken from a similar decision analysis study of patients with acute myeloid leukemia. In terms of the timing of up-front allo-HSCT, it was set as all patients receive up-front allo-HSCT from 2 to 6 months if ATL did not progress before allo-HSCT. We used the TreeAge Pro 2016 software package for decision analysis (TreeAge Software Inc., Williamstown, MA).

Results: In all patients, up-front allo-HSCT was associated with higher LE in comparison to chemotherapy alone (2.26 years vs 1.75 years). Stratified into 3 groups according to the prognostic scoring system, LE of up-front allo-HSCT was higher compared to that of chemotherapy alone in the intermediate- (2.27 years vs 1.66 years) and high-risk groups (1.50 years vs 0.91 years). The estimated survival curve depicted by TreeAge showed the superiority of up-front allo-HSCT as shown in Figure 1A-D. The Monte Carlo simulation showed that the probability of superiority of up-front allo-HSCT was 100% in all patients, 97.1% in the low-risk group, 100% in the intermediate-risk group, and 100% in the high-risk group in terms of LE, and was 98.8% in all patients, 75.2% in the low-risk group, 100% in the intermediate-risk group, and 100% in the high-risk group in terms of QALE.

Summary/Conclusions: Based on decision analysis, up-front allo-HSCT was associated with higher LE and QALE in the intermediate- and high-risk groups in comparison to chemotherapy alone in patients with aggressive ATL. In the absence of prospective randomized controlled trials, our results suggest that up-front allo-HSCT for aggressive ATL is the favored treatment strategy in the intermediate- and high-risk groups.

P386

OUTCOMES OF THIOTEAPE BASED REDUCED-INTENSITY CONDITIONING VERSUS STANDARD-REBUCED-INTENSITY CONDITIONING IN ADULT PATIENTS UNDERGOING DOUBLE-UNIT CORD-BLOOD HEMATOPOIETIC STEM CELL TRANSPLANT

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Background: Cord blood transplantation (CBT) is an established alternative source for hematopoietic stem-cells in patients without matched donor. However, the most commonly used high-dose total-body-irradiation (TBI) myeloablative conditioning (MAC) results in high treatment related mortality (TRM). Non-myeloablative and reduced-intensity conditioning (RIC) have been studied to decrease TRM and provide curative chance to the elderly and those with comorbidities. However, these strategies are associated with higher relapse-rate and graft rejection. A novel-RIC using addition of thiotepa and higher dose of TBI to standard RIC has shown to result in sustained donor engraftment. Our study compares transplant-related-outcomes in patients who underwent first double-unit CBT with standard-RIC regimen of fludarabine (Flu, 200mg/m²), cyclophosphamide (Cy, 50mg/kg), and TBI (200Cy or 300CyGy) versus this standard-RIC regimen with addition of thiotepa (10mg/kg) and increased dose of TBI (60CyGy).

Aims: 1. To compare transplant related outcomes in CBT recipients who received standard-RIC (FluCyTBI) to those who received novel-RIC (FluCy with addition of thiotepa and increased dose of TBI). 2. To identify optimal conditioning regimen in patients undergoing UCT.

Methods: After IRB approval, consecutive patients undergoing CBT from 08/2009 to 08/2016 were evaluated and data retrospectively abstracted. Patient selection, graft-versus-host disease prophylaxis and transfusions were per institutional standards and conditioning regimens were compared as described. Results: 1. The 99 patients who underwent allogeneic double-CBT, 52 received standard-RIC and 47 received novel-RIC. Median age at transplant was 67 years (range, 24-74) and 54 years (range, 25-67) in standard-RIC and novel-RIC cohort respectively. Acute myeloid leukemia was the major indication for transplant in both cohorts. Median hematopoietic stem-cell transplant comorbidity-index (HSCT-CI) was 3 (range, 0-6) and 1 (range, 0-5) in standard-RIC and novel-RIC groups respectively. Four patients suffered engraftment failure (2 in each cohort). Median neutrophil engraftment was 13 days (range, 6-42) and 21 days (range, 12-43) while median platelet engraftment was 37 days (range, 26-70) and 38 days (range, 24-74) in standard-RIC and novel-RIC groups respectively. Fifty-three suffered acute-GVHD which occurred in 21 (40%) patients (grade 2-4: n=15, 29%; grade 3-4: n=4, 8% in standard-RIC group and in 32 (66%) patients (grade 2-4: n=29, 62%; grade 3-4: n=5, 11%) in novel-RIC group. Chronic-GVHD (cGVHD) occurred in 18 patients (n=7, 14% in standard-RIC; n=11, 23% in novel-RIC group). The one-year cumulative incidence of relapse was 36% (n=15) in standard-RIC while it was 15% (n=5) in novel-RIC cohort. Median relapse free survival (RFS) was significantly improved in novel-RIC cohort compared to standard-RIC (HR, 0.32, CI:0.11- 0.76, p=0.01). Median RFS was 29 months in standard-RIC cohort while median RFS was not reached in novel-RIC cohort. The one-year cumulative incidence of transplant related mortality (TRM) was 22% (n=10) in those who received standard-RIC while it was 16% (n=7) in those who received novel-RIC. TRM was not significantly different between the standard-RIC and novel-RIC cohorts. Median follow-up in standard-RCI cohort was 9.3 months (range, 0.16- 79) and 13 months (range, 1.4- 36) in novel-RCI cohort. The overall survival (OS) was significantly better in novel-RCI cohort compared to standard-RCI (HR 0.49, CI: 0.25- 0.94, p= 0.03). Median OS was 17 months in standard-RIC cohort while median OS was not reached in novel-RIC group (Figure 1).

Summary/Conclusions: In our study, RIC consisting of FluCy with addition of thiotepa and increased dose of TBI in patients undergoing double-cord UCT was associated with improved OS and improved RFS without increase in TRM as compared to standard RIC. While older and more comorbid patients might experience increased TRM with the thiotepa based regimen, these data suggest that consideration of this regimen may be appropriate in fit, older patients.

P387

INTERFERON-A IS EFFECTIVE FOR TREATMENT OF MINIMAL RESIDUAL DISEASE IN PATIENTS WITH ACUTE LEUKEUMA AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Post-transplant relapse is a major cause of transplant failure. Because impending relapse can be indicated by minimal residual disease (MRD) after allogeneic hematopoietic stem-cell transplantation (allo-HSCT), MRD-directed intervention may be a reasonable option for relapse prophylaxis.

Aims: We investigated the efficacy of MRD-directed interferon-a (IFN-a) treatment in acute leukemia patients who were positive for MRD after allo-HSCT.
Methods: A total of 107 patients who were MRD-positive after allo-HSCT were enrolled. MRD-positive status was defined as positivity for leukemia-associated aberrant immune phenotypes or positivity for Wilms' tumor gene 1 in a single bone marrow sample. Recombinant human IFN-α-2b injections were administered subcutaneously 2–3 times per week for 6 months.

Results: The 2-year cumulative incidence of severe acute and chronic graft-versus-host disease (aGVHD and cGVHD) was 47% (43-51%) and 20% (15-27%), respectively. Multivariate analysis identified active myeloid leukemia at SCT as a significant risk factor for late relapse. Non-relapse mortality (NRM) occurred in 13% (11-16%) and 21% (15-28%), respectively. Advanced age was the only risk factor for late relapse. Non-relapse mortality (NRM) occurred in 13% (11-16%) and 21% (15-28%), respectively. Advanced age was the only risk factor for late relapse. Non-relapse mortality (NRM) occurred in 13% (11-16%) and 21% (15-28%), respectively. Advanced age was the only risk factor for late relapse.

Summary/Conclusions: These data confirmed that MRD-directed IFN-α treatment is effective for patients who were MRD-positive after allo-HSCT.

P389

IMPACT OF AZACITIDINE PRETREATMENT ON OUTCOMES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH HIGH-RISK MYELODYSPLASTIC SYNDROME


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Background: Myelodysplastic syndrome (MDS) is a heterogeneous myeloid stem cell disorder with ineffective hematopoiesis, dysplastic cell morphology, and a propensity for progression to acute myeloid leukemia. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only curative therapy for MDS (1). In recent years, azacitidine (AZA) has been increasingly used as pre-transplant induction therapy in high-risk MDS patients. However, the benefits of pretransplant therapy in these patients are unclear, and the optimal therapy regimen remains unknown.

Aims: We conducted a retrospective analysis to elucidate the clinical impact of pre-treatment with AZA on outcomes after allo-HSCT in high-risk MDS patients.

Methods: Clinical data were collected from the registry database of the Japan Society for Hematopoietic Cell Transplantation. We selected patients with high-risk MDS at diagnosis (IPSS intermediate 2 or high), aged 16 years or older, who were transplanted in their first transplantation between January 2009 and December 2014 and received AZA or best supportive care (BSC) before allo-HSCT. Patients who received conventional chemotherapy or immunosuppressive therapy prior to allo-HSCT were excluded. We compared overall survival (OS), relapse, non-relapse mortality (NRM), and hematopoietic recovery after allo-HSCT in both groups. Our study was retrospective.

OS was estimated by the Kaplan–Meier method, and a log-rank test was used for comparisons. Relapse and NRM were considered competing risk events and were compared using Gray’s test. The cumulative neutrophil and platelet recoveries were also compared by Gray’s test, considering death without these events as a competing risk. In a multivariable analysis, the Cox proportional hazard model and Fine-Gray methods were used for OS and cumulative incidence of relapse and NRM and hematopoietic recovery, respectively, using the following variables: age, gender, performance status at transplantation, marrow blast at diagnosis, cytogenetic risk, donor source, donor-recipient gender matching, and previous chemotherapy.

Results: Of the 485 patients, 161 patients (33.2%) received AZA and 324 patients (66.8%) received BSC before allo-HSCT. The median age was 60 (18–70) and 56 (18–74) years, respectively (P=0.002). A higher proportion of BSC patients received cord blood transplantation (P=0.005). Bone marrow derived from related donors (n=161) and autologous transplantation (n=15) were not included in this analysis. Differences in the median age between the AZA and BSC groups. In multivariate analysis, AZA and BSC showed comparable OS (HR, 1.16; P=0.31), relapse (HR, 1.13; P=0.50), NRM (HR, 0.92; P=0.64), neutrophil engraftment (HR, 1.01; P=0.89), and platelet engraftment (HR, 1.07; P=0.59).

Conclusion: We confirmed that pretransplant AZA and BSC provide similar outcomes of allo-HSCT in high-risk MDS patients. Further analysis is needed to clarify the role of pretransplant therapy in high-risk MDS and to identify the subset of patients who may benefit from pretransplant AZA.
LOW-DOSE DECITABINE IMPROVES PLATELET RECOVERY IN PATIENTS WITH ISOLATED THROMBOCYTOPENIA AFTER HSCT

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Background: Isolated thrombocytopenia is a common complication of hematopoietic stem-cell transplantation (HSCT), which was defined as consistent low platelet counts with recovery of the other two cell lines after transplantation. This status leads to an increased risk of life-threatening hemorrhage, frequent requirements of platelet transfusion and extended hospital stays, representing a challenging clinical problem. Previous studies have demonstrated that decitabine, a hypomethylating agent, may increase platelet counts by promoting megakaryocyte maturation and platelet release in mouse model.

Aims: In order to investigate the role of decitabine in patients after HSCT suffering from isolated thrombocytopenia, we conduct a clinical trial to validate this effect in post-HSCT setting.

Methods: We performed a prospective open-label study to evaluate the treatment of low-dose decitabine in patients with hematological malignancies who received allogeneic HSCT and suffered from isolated thrombocytopenia. The inclusion criteria were: (1) Platelet count ≤30 × 10^9/L persistently at day 60 post-HSCT or later; (2) Recovered neutrophil and hemoglobin; (3) Full donor chimerism; and (4) No response to conventional treatments for a duration of at least 4 weeks. Patients with malignancy relapse, active infections, uncontrolled graft-versus-host disease, severe organ damage or transplant-related thrombosis were excluded. From July 2013 to July 2016, 38 patients were randomly assigned into either the control group to receive conventional treatment only, or the test group to receive additional decitabine (15mg/m^2, intravenously daily for 3 consecutive days).

Results: Major response was observed in 16 out of 19 patients (84.2%) in decitabine group, with a median time of 22 days to achieve platelet transfusion-independence. Two patients (10.5%) showed a minor response and 1 patient (5.3%) failed. In contrast, 3 out of 19 patients in the control group (15.8%) showed a major response, 2 patients (10.5%) showed a minor response, 14 patients (73.7%) did not show any improvement, of which 1 patient died of severe hemorrhage in week 5. For bone marrow morphological analysis, all 38 patients showed low levels of megakaryocytes at week 0. However, the megakaryocyte counts in decitabine group were significantly increased at week 4, while no significant difference was recorded in control group. After decitabine treatment, we did not observe a change in anti-platelet antibodies levels and T cell subsets ratios. However, reactive oxygen species (ROS) and megakaryocyte counts increased in the test group. No considerable myelosuppression, febrile neutropenia, and nonhematologic toxicities associated with the treatment were observed.

Summary/Conclusions: Our data showed an encouraging efficacy of decitabine in patients after HSCT suffering from isolated thrombocytopenia, and suggested to remarkably increased megakaryocyte counts. Decitabine may improve isolated thrombocytopenia via regulating ROS and megakaryocyte reconstitution.

Thalassemia

P391

QUANTITATIVE PROTEOMICS OF PLASMA EXTRACELLULAR VESICLES TO IDENTIFY NOVEL BIOMARKERS OF CLINICAL SEVERITY FOR HBE/B-TALASSEMIC PATIENTS

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Background: Hemoglobin (Hb) E/B-thalassemia has a wide spectrum of clinical manifestations that cannot be explained purely by its genetic background. Extracellular vesicles (EV) are one factor that may indicate and/or contribute to disease severity because there is an observed increase in EV release due to the increased oxidative stress in thalassemic erythrocytes.

Aims: This study aims to explore the differences in protein composition and abundance between circulating EV from HbE/B-thalassemic patients and normal individuals.

Methods: 15 HbE/B-thalassemia patients and 15 matched-controls from Thailand were fully consented and recruited for this study. Pooled EVs isolated from five thalassemic samples were compared to pooled EVs from five matched controls using a Duplex-Tandem Mass Tag (TMT) mass spectrometry (TMT-MS) analysis. This experiment was repeated three times in total, using different patient and control samples to identify consistent alterations of protein expression in EVs. Finally, protein differences were also confirmed using Western blotting.

Results: The total proteins identified across the three experimental TMT-MS datasets ranged from 1.764 to 2.534 proteins. While restricted to proteins that contained more than one unique peptide, the range of proteins was reduced to 685 to 1,272 proteins. Many proteins were previously reported EV constituents. 19 proteins were consistently increased in patient samples compared to controls across all data sets. The majority of these proteins were chaperone proteins and antioxidant enzymes. Alpha Hemoglobin Stabilizing Protein (AHSHP) had the highest increase of between 31 to 47-fold. Other proteins that exhibited increased abundance in thalassemic circulating EV included catalase, superoxide dismutase, T-complex proteins, heat shock protein 70 and ferritin light chain. Importantly, the heme scavenger and plasma proteins – haptoglobin and hemopexin were observed to be consistently decreased in patients’ EV across all data sets. Immunoblotting results corroborated the TMT-MS findings.

Summary/Conclusions: We have successfully identified consistent alterations in protein expression levels between EV generated by HbE/B-thalassemic patients and normal individuals. These findings may potentially lead to the development of a prognostic marker, and therefore may improve the therapeutic outcome for the patients suffering from thalassemia.
and in the percentage of circulating erythroblasts; (iv) the increase in β Thal red cell survival. RO4917838 induced a significant reduction in extramedullary erythropoiesis as well as in the amount of insoluble alpha chain aggregates in circulating red cells. It is of note that in β-Thal sorted erythroblasts we found a reduction in HRI and in phospho-elf2a, inducing a reduction in free heme, which shall result in the activation of HRI, in RO4917838 treated β -Thal mice (10 mg/kg/d, 6 weeks). Finally, in β-Thal mice treated with RO4917838 (4 weeks at 30 mg/kg/d) a reduction in liver and spleen iron-overload was identified, which was associated with increased hepatic iron expression.

Summary/Conclusions: Our data suggest that RO4917838 ameliorates anemia and ineffective erythropoiesis by reduction of heme biosynthesis in a mouse model for β-thalassemia. RO4917838 is a potential, novel therapeutic approach for the treatment of anemia in patients affected by beta-thalassemia.

P393

MAY MUTATIONS IN THE KLF1 GENE HAVE WORSENING EFFECTS ON THE BETA THALASSEMA PHENOTYPE

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1Dep. Molecular Medicine and Medical Biotechnology, University of Naples Federico II; 2USC Genetica Medica, AORN A. Cardarelli; 3UOC Patologia Clinica, P.O. Pellegrini, ASLNA1; 4UOSS Malatte rare del globo rosso, AORN A. Cardarelli, Naples; 5UOCC Pediatría-TIN, P.O. Umberto I, Nocera Int., Italy

Background: Kruppel-like factor 1 (KLF1) is a pleiotropic erythroid transcriptional factor that plays a key role in erythropoiesis (Siatecka M, Blood 2011; 118: 2044-521). Accordingly, KLF1 mutations have been found to be responsible for a variety of hematological disorders. KLF1 also contributes directly or indirectly to regulate the expression of genes in the beta-globin gene cluster (Wayne JS et al Int. J. Lab. Hem. 2015; 37: 78-84). It has been reported that mutations leading to KLF1 haploinsufficiency result in a decrease in adult β-globin (HbF) levels with ameliorative effects on the severity of beta-thalassemia (Liu D. et al. Blood 2014; 124: 803-811; Perkins A. et al. Ann. Hematol 2013; 92: 53-58) and two novel mutations (c.-148 G>A) in the proximal promoter region, F182L and M39L) (Radmilovic M. et al. Blood 2013; 122: 803-811; Perkins A. et al. Ann. Hematol 2013; 92: 53-58) were identified, which was associated with increased hepatic iron expression.

Summary/Conclusions: Our data suggest that RO4917838 ameliorates anemia and ineffective erythropoiesis by reduction of heme biosynthesis in a mouse model for β-thalassemia. RO4917838 is a potential, novel therapeutic approach for the treatment of anemia in patients affected by beta-thalassemia.

P393

SECONDARY SOLID TUMORS FOLLOWING HEMATOPOIETIC CELL TRANSPLANTATION FOR THALASSEMIA MAJOR

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1Fondazione G. Monasterio ONR-Regione Toscana, Pisa, 2Bone Marrow Transplant Center, Pescara, 3University of L’Aquila, L’Aquila, 4Tor Vergata University, Roma, 5ARNAS Civico “Benferratelli-Di Cristina”, Palermo, 6AORN A. Cardarelli, Napoli, Italy

Background: Secondary solid tumors (SST) have been described after HCT, in particular for patients affected by hematologic malignancies. There is limited information about the incidence of SST following HCT for thalassemia major (TM).

Aims: The aim of this study was to determine the incidence of SST in 134 patients with TM who receive HCT in our Center between 1983 and 2013.

Methods: 117 patients survived more than 3 years after HCT and were enrolled in the study. Of them, 57 were males and 60 females. Their median age at time of HCT was 10 years (1-29). As conditioning regimen, they received Busulfan (14 mg/Kg) and Cyclophosphamide (200 mg/Kg). The GvHD prophylaxis included Cyclosporine and Methotrexate. All patients received bone marrow cells from an HLA identical donor.

Results: At time of this report, 112 patients were cured, whereas 5 patients rejected their graft and are now under regular transfusion treatment. Overall, the median follow-up after HCT was 24 years (3-34). Seven patients developed a malignancy (6 males, median age 10 years) 10 years after HCT including 2 cancers of the tongue, 1 oral squamous cell carcinoma, 1 colorectal cancer, 1 thyroid carcinoma, 1 carcinoma of the uterine cervix, and 1 parotid carcinoma. The 30-yr cumulative incidence (CI) of developing SST was 10±0.17%. All patients underwent surgical resection of the tumor and in addition of them received chemotherapy and/or radiotherapy. Of relevance, 3 patients with cancer of the oral cavity were affected by severe chronic GVHD with buccal cavity involvement. Two patients (1 with parotid and 1 with tongue carcinoma) died of tumor progression and are living. We compared these results with 2 case control populations. First of all, we investigated the occurrence of solid tumors in the 117 individuals (64 males, median age 10 years at time of marrow donation), who served as stem cell donors for HCT. One donor developed breast cancer 29 years after marrow donation at age of 38. The 30-yr CI of developing solid tumor for donors was 4.5±0.21% with a statistically significant difference (p=0.03) as compared to that of transplanted patients. The second case control population consisted of 134 individuals (64 males, median age 10 years at time of marrow donation), who served as stem cell donors. Notably, among the transplanted patients we didn’t observe any case of HCC, which is one of the most frequent solid tumor in nontransplant TM patients, whereas we observed 4 cases of head/neck cancers. In our series, cGVHD seems to be a strong risk factor in the development of new solid tumors. Patients with cGVHD, especially those with involvement of the oral cavity, must receive a very long care monitoring and surveillance in order to prevent the development of secondary cancers.

P395

VALIDATING A NOVEL CAPILLARY ELECTROPHORESIS: THE MOST SUITABLE PLATFORM FOR THE NATIONAL NEWBORN SCREENING DEVELOPED IN A REGION WITH HIGH PREVALENCE OF THALASSEMA AND HEMOGLOBINOPATHIES

T. Sukangpleng1, S. Riolueang1, J. Korchuenjit1, W. Korchuenjit1, J. Poolam2, V. Virapaksa1,3
Background: Newborn screening program for thalassemia (thal) and hemoglobinopathies (NBS-Hbs) is crucial for early detecting patients with severe hemoglobinopathies (Hb variants) e.g. sickle cell anemia (Hb SS). NBS-Hbs has been incorporated into a routine neonatal service in several developed countries. However its role on early detection other forms of globin disorders remains unclear. Moreover, NBS-Hbs can detect several types of thalassemia and Hb variants carriers. This application could be useful for the national prevention and control programs especially in the countries where these conditions are highly prevalent especially β-thal major. Hb E/β-thal and Hb Bart’s hydrops fetalis (caused by α∗-thalassemia). Recently a new capillary electrophoresis (CE) has been developed specifically for NBS-Hbs. However there is a limited data on validation of this technology on detecting several types of thalassemia and Hb variants found in Southeast Asia.

Aims: To evaluate and validate a new CE system to screen globin disorders in newborn to initiate the national NBS-Hbs for Thailand.

Methods: After informed consent, 1,213 blood samples of 2-day old newborns were collected by heel prick puncture into 5-dried blood spots. After elution, dried blood samples were analyzed by Capillaries 2 NEONAT FAST® (SEBIA, Evry, France). All samples were also extracted for DNA and genotyped by our extensive PCR based panel to detect >98% of abnormal globin alleles found in Thailand using α-thal GAP-PCR, α-thal ARMS-PCR, β-thal ARMS-PCR, and PCR-RFLP for Hb E. We compared CE data with each globin genotypes and use a ROC curve to set up new diagnostic criteria using% Hbs from CE for future cases.

Results: Identification of Hb Bart’s provided 100% of sensitivity, specificity, and accuracy in most individuals with α-thal. Using ROC analysis, we proposed different cut-off values of Hb Bart’s to differentiate Hb H disease, α∗-thal and non-deletional α-thal traits; ≥7.40%, ≥0.85%, ≥0.45%, respectively with excellent accuracy (Table 1). Interaction of Hb E with these α-thal genotypes has no effect on these cut-off values (Table 1). However, there was a limitation to identify deletional α-thal trait using CE by the level of Hb Bart’s ≥0.10% (detectable level). A cut-off level to distinguish Hb EE from Hb E trait was suggested at ≥0.7%. Two patients with Hb E/β-thalassemia were identified through this study with different CE pattern from Hb EE. 11 β-thalassemia traits were identified and they had a lower level of Hb A as compared to their gestational age (GA). Sex matching controls with normal β-globin genotypes (n=148). We recommend Hb A level ≤10.35%; a cut-off to primarily consider for β-thalassemia carrier followed by molecular analysis.

Table 1.

Summary/Conclusions: This newborn CE platform showed a high efficiency for detecting several types of thalassemia and Hb variants in particular α-thal, β-thal and Hb E using cut-off levels of each Hb species described herein. Besides early detecting of Hb S, we can now apply this NBS into a routine service in order to early detect Hb H disease. Hb E/β-thalassemia and the majority of common thalassemia carriers. This NBS-Hbs approach can reinforce the current program on prevention and control for severe thalassemia syndromes in many developing countries including Thailand where these conditions are highly prevalent especially β-thal major. Hb E/β-thal and Hb Bart’s hydrops fetalis (caused by α∗-thalassemia). Recently a new capillary electrophoresis (CE) has been developed specifically for NBS-Hbs. However there is a limited data on validation of this technology on detecting several types of thalassemia and Hb variants found in Southeast Asia.

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Results: Identification of Hb Bart’s provided 100% of sensitivity, specificity, and accuracy in most individuals with α-thal. Using ROC analysis, we proposed different cut-off values of Hb Bart’s to differentiate Hb H disease, α∗-thal and non-deletional α-thal traits; ≥7.40%, ≥0.85%, ≥0.45%, respectively with excellent accuracy (Table 1). However, there was a limitation to identify deletional α-thal trait using CE by the level of Hb Bart’s ≥0.10% (detectable level). A cut-off level to distinguish Hb EE from Hb E trait was suggested at ≥0.7%. Two patients with Hb E/β-thalassemia were identified through this study with different CE pattern from Hb EE. 11 β-thalassemia traits were identified and they had a lower level of Hb A as compared to their gestational age (GA). Sex matching controls with normal β-globin genotypes (n=148). We recommend Hb A level ≤10.35%; a cut-off to primarily consider for β-thalassemia carrier followed by molecular analysis.

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Summary/Conclusions: This newborn CE platform showed a high efficiency for detecting several types of thalassemia and Hb variants in particular α-thal, β-thal and Hb E using cut-off levels of each Hb species described herein. Besides early detecting of Hb S, we can now apply this NBS into a routine service in order to early detect Hb H disease, Hb E/β-thalassemia and the majority of common thalassemia carriers. This NBS-Hbs approach can reinforce and leverage our current program on prevention and control for severe thalassemia syndromes in our region. Moreover, due to population migration from The East to the West, our new diagnostic guideline by CE could be useful and applicable for existing NBS programs currently available in several European countries.

P396

TRANSIENT ELASTOGRAPHY IN NON TRANSFUSION DEPENDENT THALASSEMIA: A SUCCESSFUL TOOL TO ASSESS AND MONITORING LIVER FIBROSIS

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Background: The introduction of close monitoring, regular blood transfusions and systematic iron chelation in the management of thalassemia have significantly changed the clinical phenotype of the patients and improved their survival. The patients, who have benefited of the current therapeutic regimen, are now reaching middle age, and they have started to manifest liver problems more commonly seen in older people. Recent observations suggest of an increased incidence of malignancies in the aging group of thalassemic patients.

Aims: The purpose of this study is to determine the longitudinal changes in the incidence of malignancies, along with possible correlations to different aspects of their disease.

Methods: A retrospective study in the largest Thalassemia Unit in Greece has been conducted spanning an observation period from 2001-2016. The occurrence and type of cancer, as well as history on transfusion dependence, liver failure, HCV infection and chelation therapy were recorded and analyzed. Statistical analysis was performed using the SPSS software package, v. 20. A p-value <0.05 was considered statistically significant.

Results: Records from 591 patients (338 with thalassemia major and 253 with thalassemia intermedia) were evaluated. 27 patients (11 males and 16 females)
Results:

4 weeks in NC.

Patients with LPI ≥0.6 µM, SF ≥1000 µg/L or TSAT ≥70% in each study arm was 8 months for both DFP-treated and for NC children. The percentage of dose (50 mg/kg/day) or no chelation (NC). Median age at 1st transfusion ≥400 μg/L or transferrin saturation (TSAT) ≥70% or labile plasma iron (LPI) ≥0.2 µM were randomized to start deferiprone (DFP) at a sub-therapeutic dose (50 mg/kg/day) or no chelation (NC). The utilization of different types of chelation changed throughout the years, according to the availability of the chelating agents, we analyzed separately, the patients that developed malignancies in the period after 2010 when longitudinal exposure to all three chelators can be assumed. Even though the results showed a difference (p=0.027) between the different groups with 47.1% of those patients receiving DFX at the time of the diagnosis compared to 27.1% receiving DFP and to 11.8% receiving DFO, this distribution reflects the overall distribution of chelator usage during that period. Apart from the incidence, there was no statistical significant difference between TD and NTD patients with cancer regarding the gender, age and year of diagnosis. The overall cancer mortality rate was 48.0%, but varied significantly with the type of cancer with liver cancer and hematological malignancies having a mortality of 66.0%. Overall only 2% of the deaths occurring in our group of patients were attributed to cancer.

Summary/Conclusions: This retrospective study has confirmed the increased incidence of malignancies in thalassemia patients in Greece, which is, at least, partially related to the aging of this population. Based on these observations, adaptation of monitoring guidelines is essential for optimal management of thalassemic patients. Periodic screening for malignancies, especially hepatic, thyroid and hematologic, will allow early detection and timely, and thus, more efficacious treatment of the neoplasia.
(79%). Figure 1 shows the changes in iron levels. Twenty-five patients changed the chelation regimen after the baseline MRI. Globally, a worsening in cardiac iron was found in 3% of the patients while a worsening in hepatic iron in the 21% of the patients (P=0.003). The LV end-diastolic volume index and all RV volumes as well as the LV mass index were significantly lower at the FU MRI. No significant improvement in left or right global systolic function was found.

For 40 patients the prevalence of myocardial fibrosis was investigated at both baseline and FU scans. Six patients (15.0%) had myocardial fibrosis at the baseline MRI and myocardial fibrosis was detected for all of them also at the FU. The extent of myocardial fibrosis was comparable between the two scans (0.77±0.42% vs 0.79±0.51%; P=0.686). At the FU 4 new occurrences of myocardial fibrosis were detected. In patients with baseline MIO no significant correlation was found between the percentage change in cardiac iron and the changes in hepatic iron or the baseline hepatic iron.

**Methods:**

**Results:**

Dec16. Magnetic resonance monitoring in children with TM demonstrated a good control of cardiac iron overload in terms of prevention and treatment but the need for further improvement of liver iron overload.

Magnetic resonance monitoring in children with TM demonstrated a good control of cardiac iron overload in terms of prevention and treatment but the need for further improvement of liver iron overload.

Mycocardial fibrosis appears mainly multifocal, non progressive and not reversible over a 18-month period. A prompt and aggressive approach to iron overload and a chelation regimen consistent with the high iron intake and the high rate of severe liver iron overload is recommended in children.

**Summary/Conclusions:** Magnetic resonance monitoring in children with TM demonstrated a good control of cardiac iron overload in terms of prevention and treatment but the need for further improvement of liver iron overload. Myocardial fibrosis appears mainly multifocal, non progressive and not reversible over a 18-month period. A prompt and aggressive approach to iron overload and a chelation regimen consistent with the high iron intake and the high rate of severe liver iron overload is recommended in children.

**P400**

**LONG TERM FOLLOW-UP OF A COHORT OF WELL TREATED B-THALASSEmia MAjor PATIENTS BY Multi-organ r2* MAGNETic RESONANCE IMAGING**

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**Background:** The introduction of non-invasive multi-organ evaluation of iron overload by R2* Magnetic Resonance Imaging (MRI) in β-thalassemia major (TM) patients has improved the patient care allowing a more careful tailoring of iron chelation therapy.

**Aims:** We report a cross-sectional and longitudinal experience with the use of MRI-R2* heart, liver and pancreas in a cohort of well treated TM patients.

**Methods:** TM patients underwent contemporaneous assessment of pancreatic, cardiac and hepatic MRI-R2* (1.5 T GE HDx scanner) in the period Jun08-Dec16.

**Results:** 69 TM patients: 43% male, age 38±9 yrs, median number of observations/patient 6 (IQR:5-7), median number of yrs of the follow-up (f.u.) 8 (IQR:7-8). Iron chelation regimens included deferasirox (basal 30%>f.u.32%), deferiprone (basal 45%>f.u.52%), daily alternating deferasirox+deferiprone (basal 3%>f.u.6%), deferoxamine (basal 9%>f.u.6%) deferoxamine+deferiprone (basal 13%>f.u.4%). The observation at the baseline showed a positive strong correlation between R2* values of pancreas and both of liver (Rp=0.53,p<0.001), heart (Rp=0.75,p<0.001), in accordance with literature. Moreover, the ROC analysis confirms the value of 100 Hz for the pancreatic-R2* as the predictor of a cardiac R2*>50Hz, we calculated the numbers of false/true positive/negative according to the rule above. At the baseline we can observe that the number of false positive is the 14/27 (52%). The percentage increases to 91% (21/23) after f.u.: the pancreas-R2*>100Hz in 23 patients but only 2 has iron overload in the heart; the total number of patients with pancreatic-R2*>100Hz is quite the same before and after f.u. (27 compared to 23). We found no correlation between the false positive predicted and particular conditions such as impaired glucose tolerance, diabetes or adipose involution (Table 1).

**Table 1.**

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>No.</th>
<th>R2*</th>
<th>R2*&gt;50Hz</th>
<th>R2*&gt;100Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>27</td>
<td>14</td>
<td>109</td>
<td>5</td>
</tr>
<tr>
<td>Liver</td>
<td>2</td>
<td>0</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Pancreas</td>
<td>51</td>
<td>23</td>
<td>51</td>
<td>23</td>
</tr>
</tbody>
</table>

**Summary/Conclusions:** In this experience we observed that the regular multi-organ assessment of iron overload by R2* is concomitant with a reduction of the iron burden in this cohort of well treated patients confirming that is a careful method to tailoring the iron chelation therapy. However pancreatic-R2* remains above the cut-off for the prediction of cardiac iron overload, so this parameter should be considered with caution in the tuning of the chelation therapy, in order to avoid over-chelation risk. Ferritin values trend agree with R2* values confirming the reliability of this parameter. These results were obtained with a prevalent use of oral chelation regimen (90% of patients).
**Transfusion medicine**

**P401**

**DEVELOPMENT OF HTLV-1 HYPERIMMUNE GLOBULINS AGAINST HTLV-1 INFECTION**

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**Background:** Adult T-cell leukemia (ATL) is a malignant disease caused by infection with human T-lymphotropic virus (HTLV-1). The prevention of HTLV-1 infection is the most effective strategy to eradicate ATL. However, there is no effective vaccine or anti-viral agent for HTLV-1.

**Aims:** The aim of this study was to develop an effective HTLV-1 hyperimmune globulin (HTLV-IG) isolated from HTLV-1 positive carriers screened at the Japanese Red Cross.

**Methods:** We developed two in vitro and in vivo screening methods to evaluate and characterize the anti-viral effect of HTLV-1 positive plasma and HTLV-IG.

**Results:** HTLV-1 positive carriers and HTLV-1 positive plasma (PVL >4) inhibited both HTLV-1 infection and syncytia formation. We purified HTLV-IG from the HTLV-1 positive plasma (PVL >4) and evaluated its effect in a humanized mouse model. NOG (NOD.Cg-Fkdcsid Ig2tm1SugJic) mice were treated with HTLV-IG for 5 days before HTLV-1 infection. During the monitoring period up to 40 days after post-infection, HTLV-1 infection was observed in untreated infected mice, but not in HTLV-IG-treated mice. The inhibitory effect of HTLV-IG was observed at the early stage of HTLV-1 Infection. Treatment with HTLV-IG at 20 days after HTLV-1 infection had a partial inhibitory effect. HTLV-1 gp46 expression in HTLV-1 infected cells was slightly reduced and the localization of these cells was changed in each tissue after the first line of treatment. These data suggest HTLV-IG is effective at the early phase of HTLV-1 infection. We also assessed the viral safety of HTLV-1 during the HTLV-IG manufacturing process. High log reduction values of HTLV-1 were observed during the Cohn fractionation process. Virus safety was assessed by PCR based assay and in vitro infection assays. We next assess the viral safety of HTLV-1 during the HTLV-IG manufacturing process. High log reduction values of HTLV-1 can be seen during the Cohn fractionation process. Virus safety was assessed with PCR based assay and in vitro and vivo infection assay.

**Summary/Conclusions:** These data suggest HTLV-IG is effective and safe for the prevention of HTLV-1 infection.

**P402**

**THE COMPARISON OF TUMOR CELLS IN THE APHERESIS MATERIAL DOES NOT PREDICT THE RESPONSIVENESS OF MULTIPLE MYELOMA PATIENTS TO AUTOLOGOUS TRANSPLANTATION**

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**Background:** The use of high dose of chemotherapy followed by autologous stem cell transplantation (ASCT) has improved the prognosis of patients with multiple myeloma (MM) and plasma cell dyscrasia. However, there is controversy over the effect of infusion of atypical plasma cells (PC) on the apheresis product.

**Aims:** To analyze whether in MM malignant plasma cell reinfusion could negatively affect responses to ASCT.

**Methods:** Patients (n=114) undergoing ASCT (n=120) for MM between June 2003 and February 2016 were enrolled in a retrospective study to analyze the prognostic value of aberrant (CD38++CD138+CD19-CD45weak) to normal phenotype (CD38++CD138+CD19+CD45+) plasma cells (A/T PC ratio) in the autograft by flow cytometry. The Durie-Salmon stage at diagnosis, response of disease to induction treatment, biological parameters, pre-ASCT percentage of PC in bone marrow and at day +100, and the mobilization (G-CSF) response (0.08 vs 0.08 ×10^6/kg). There was no difference between the type of mobilization (G-CSF vs chemotherapy+G-CSF) and the degree of apheresis contamination (median A/T PC ratio 0.5 vs 0.8; P=0.86). There was a statistical trend between the degree of infiltration of PC in the bone marrow before ASCT and the detection of atypical PC in the graft (r=0.006). At day +100, 94% of patients with CR or VGPR to induction therapy maintained the response, and 49% of patients in PR, SD or PD achieved post-ASCT CR or VGPR (p=1.24^-7). There was no association between the content of atypical PC in the graft and the response to day +100. However, the percentage of pre-ASCT PC in the bone marrow was significantly related to the response at day +100 (CR or VGPR vs PR, SD or PD), p=0.003, as well as the pre-ASCT monoclonal component (p=4.03^-7).

**Summary/Conclusions:** Infusion of PC with atypical phenotype does not appear to affect the response at day +100 following ASCT, in patients with MM or plasma cell dyscrasia. Conversely, the quality of response to induction therapy was significantly associated to 100-day outcome after transplantation. These data support that in vivo persistent residual cells, but not those being infused with the graft, are the main source of relapse in MM.

**P403**

**EVALUATION OF THERAPEUTIC PLASMA EXCHANGE AT A TERTIARY LONDON HOSPITAL**

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**Background:** Therapeutic plasma exchange (TPE) is used to treat a number of haematological, renal and neurological conditions. Pathogenic antibodies or other plasma molecules are removed, and plasma volume is replaced with fluid, albumin and albumin solution (HAS) is usually preferred, except in cases of Thrombotic Thrombocytopenic Purpura (TTP) and related conditions. TPE may result in dilutional coagulopathy, and reactions such as hypersensitivity can occur. The British Society for Haematology (BSH) published a 2015 guideline to assist the use of TPE in UK clinical practice, providing evidence-based indications and recommended schedules.

**Aims:** To evaluate the use of elective TPE at a large tertiary London hospital, compare clinical practice against BSH guideline recommendations, and explore the effect of TPE on coagulation test results.

**Methods:** Data was collected prospectively over a 2 month period, using patient notes and electronic transfusion records. A data collection form recorded the indication, treatment schedule, replacement fluid, complications, the presence of a written treatment plan, and frequency and results of coagulation testing.

**Results:** 24 plasma exchanges took place over the period of data collection; there were no cases of TTP. Adherence to BSH was variable; although most cases (88%) had an evidence-based clinical indication for TPE, just 4% had a full written treatment plan, and only 17% of courses followed recommended scheduling. 75% of patients had received at least one prior course, some outside guideline indications for repeat courses. Most patients (83%) initially received appropriate replacement fluid (HAS), but the remainder received FFP at some point during TPE, with 42% receiving Solvent Detergent FFP. In 17% of patients this fluid change was due to a reaction, but for the remainder it was due to dilutional coagulopathy. The guidelines recommend fibrinogen monitoring, and although most patients had baseline measurement (75%), subsequent testing showed wide variation. Fibrinogen levels should show some correction by the next day but usually still abnormal. A prolonged APTT and PT was also seen in most patients immediately following TPE, which almost always corrected by the next day.

**Table 1.**

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Median age, yr (range)</th>
<th>Male (%)</th>
<th>Plasma cell dyscrasia, n (%)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Multiple myeloma, IgG</td>
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<td>- Multiple myeloma, IgA</td>
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<td>- Bence-Jones multiple myeloma</td>
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<td>- Plasma cell leukemia</td>
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<td>- Other: Non-secretory myeloma</td>
</tr>
<tr>
<td></td>
<td>60 (36-70)</td>
<td>55 (45.8)</td>
<td>66 (55.0)</td>
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<td></td>
<td></td>
<td></td>
<td>28 (23.3)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>15 (12.5)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>5 (4.2)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>6 (5.0)</td>
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<table>
<thead>
<tr>
<th>Grade</th>
<th>Stage I</th>
<th>Stage II</th>
<th>Stage III</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>10 (8.3)</td>
<td>36 (30.0)</td>
<td>64 (53.3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Response to induction treatment before ASCT, n (%)</th>
<th>Complete response</th>
<th>Very good partial response</th>
<th>Partial response</th>
<th>Stable disease</th>
<th>Progressive disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>36 (30.0)</td>
<td>19 (15.8)</td>
<td>55 (45.8)</td>
<td>9 (7.2)</td>
<td>1 (0.8)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mobilization regimen, n (%)</th>
<th>G-CSF</th>
<th>Chemotherapy and G-CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>87 (72.5)</td>
<td>33 (27.5)</td>
</tr>
</tbody>
</table>
**Summary/Conclusions:** TPE use was generally compliant with BSH guidelines regarding clinical indication and initial replacement fluid. However many patients were changed from HAS to FFP due to measured or predicted coagulopathy. This is a recognised complication of TPE, and the guidelines suggest that if possible, TPE can take place on alternate days to ameliorate this. Fluid change to FFP is recommended only for those at increased haemorrhagic risk. Almost all the TPEs in our study took place over 3 to 5 subsequent days, reflected in the high frequency of hypofibrinogenemia. The optimum frequency of fibrinogen monitoring, and the level that should prompt change to the TPE schedule, require further exploration. The following are planned to enhance adherence to BSH guidelines and improve patient care: 1. Documented treatment plans with clinical indication, proposed treatment schedule, replacement fluid. 2. Local trust guidelines to include recommended TPE schedules, agreed parameters to monitor response, frequency of fibrinogen monitoring, common complications and their management. Where possible, TPE should take place on alternate days to reduce dilutional coagulopathy. 3. Education of staff involved with service provision, and strengthening of the role of apheresis nurse as lead.

**Results:** Of 141 participants who took part in the survey, 31% (43) had been qualified for less than two years and 47% (65) were consultants. Specialties included Surgery, Anesthesitics, Internal Medicine, Hemato-Oncology and Intensive Care. 60% (84) had prescribed blood within the last month. Despite only 51% (72) awareness of the NICE guidelines, a significant majority (73%, 103) selected the correct Hb threshold of ≤70g/L for transfusion in patients without acute coronary syndrome with a medical indication. The threshold of <50g/L was selected by 35% (50), and 7% (10) did not check transfusion Hb at all. Ferritin measurement was inconsistent with only 47% (66) routinely measuring this prior to transfusion, and only 31% (44) aware that a ferritin result over 30 days old should be rechecked. This highlighted potentially inadequate identification of iron deficiency anemia. In addition only 40% (57) were aware of the existence of a hospital anaemia clinic for referral. When reflecting on consent methods, 96% (135) of participants expressed need for indication and justification of transfusion, and 90% (127) gave an opportunity to ask questions and ensured the patient was content to proceed. Provision of written information was poor (26%, 37) and only 55% (78) recorded the discussion in patients’ notes. Exploring barriers to consent, 24% (32) expressed difficulty in obtaining a patient information leaflet, and issues relating to lack of time and confidence were 16% (22) and 9% (12) respectively.

**Summary/Conclusions:** Although the majority of participants expressed awareness of the NICE guidance, knowledge was not reflected in subsequent questions. The survey allowed simultaneous assessment of knowledge and provision of key information as a factsheet. Almost all participants felt that completion of the survey had been helpful, and as a tool to reach a highly mobile group, the survey is a constructive and supportive method to facilitate implementation of national guideline by medical staff. We were also able to identify areas that need further development including the clinical referral pathway for the anaemia clinic and improving the availability of patient information leaflets on hospital wards. At present we are planning to introduce a hospital transfusion committee which will lead on disseminating to all hospital staff, and carrying out structured case based discussion sessions with junior doctors to enhance knowledge and confidence.

**P404**

**A COMPREHENSIVE PROTEOMICS STUDY ON PLATELET CONCENTRATES: PLATELET PROTEOME, STORAGE TIME AND MIRASOL PATHOGEN REDUCTION TECHNOLOGY**

**V. Salunkhe1,*, F. van Alphen2, I.M. De Cuyper1, B. Nota1, C. van der Zwaan2, M. Healey1, M. Sivakumaran2, M. Platt1**

**Background:** Platelet concentrates (PCs) represent a blood transfusion product with a major concern for safety as their storage temperature (20-24ºC) allows bacterial and fungal growth, and their maximum storage time period (less than a week) precludes complete microbiological testing. Pathogen reduction technologies (PRTs) provide an additional layer of safety to the blood transfusion products from known and unknown pathogens (such as bacteria, viruses and parasites). In this context, Mirasol PRT is a technology that reduces viral infectivity of PCs during storage. However, those significant changes at the proteome level were specifically related to the functional aspects previously described to identify proteins using MaxQuant/Perseus software platform.

**Methods:** We present comprehensive proteomics data analysis of control PCs and PCs treated with Mirasol PRT at storage day 2, 6 and 8. Our workflow was set to perform proteomics analysis using a gel-free and label-free quantification (LFQ) approach. Semi-quantification was based on LFQ signal intensities of identified proteins using MaxQuant/Perseus software platform.

**Results:** We identified marginal differences between Mirasol PRT and untreated PCs during storage. However, those significant changes at the proteome level were specifically related to the functional aspects previously described to identify proteins using MaxQuant/Perseus software platform.

**Summary/Conclusions:** In summary, semi-quantitative proteomics allows to discern between proteome changes due to Mirasol PRT or PSL, and proves to be a methodology suitable to phenotype platelets in an unbiased manner, in various physiological contexts.

**P405**

**USE OF A SURVEY TO ASSESS AND IMPROVE ADHERENCE TO UK BLOOD TRANSFUSION GUIDELINES IN A HOSPITAL SETTING**

**D. Warcel1,*, R. Moll1, A. Li1**

**1Haematology, Royal Free Hospital, London, United Kingdom**

**Background:** UK guidelines to provide evidence-based support for decisions to transfuse packed red cells were published in 2015 by NICE (National Institute for Healthcare and Excellence). The guidelines specified hemoglobin (Hb) targets for transfusion, use of single unit transfusion to avoid over-transfusion, information provision to patients for informed consent, and avoidance of pre-operative transfusion by timely identification of iron deficiency for referral through an anaemia pathway. A local baseline audit of NICE compliance at our London teaching hospital showed low overall compliance with these recommendations.

**Aims:** To determine knowledge amongst the prescriber group of transfusion guidelines for stable patients, to gain insight into current patterns of decision-making for transfusion and to impart knowledge of the key NICE guidance.

**Methods:** An online survey, designed to both evaluate and inform participants, was targeted at doctors of different training grades and specialties during a two week period. The outcomes of this are being used to guide further training.
Front-line combinations in multiple myeloma and amyloidosis

S407

QUADRUPELT VS SEQUENTIAL TRIPLET INDUCTION THERAPY FOR MYELOMA PATIENTS: RESULTS OF THE MYELOMA XI STUDY


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Background: Combining anti-myeloma induction therapies limits the impact of clonal heterogeneity on resistance to therapy, maximising response and associated clinical outcomes. Triplet combinations induce deeper, longer remissions than doublets and those containing an immunomodulatory agent, a proteasome inhibitor (PI) or both are the current standard of care in Europe/US. Potential approaches to further improve outcomes include response-adapted induction, treating suboptimal responders with sequential treatment using an agent with a different mechanism of action, or intensifying induction for all patients by the use of quadruplet combinations upfront.

Aims: The UK NCRI Myeloma XI trial is a large, phase III study comparing, in transplant eligible (TE) patients, the induction quadruplet carfilzomib, cyclophosphamide, lenalidomide and dexamethasone (KCRD) to the sequential strategy of triplet immunomodulatory combinations (with thalidomide or lenalidomide) followed by additional pre-transplant consolidation with PI triplet therapy for those with a suboptimal response.

Methods: In 2013, the TE pathway of the Myeloma XI study was amended to include KCRD given in 28 day cycles (carfilzomib 36mg/m2 IV d1-2,8-9,15-16 (20mg/m2 #1d1-2), cyclophosphamide (cyclo) 500mg PO d1,8, lenalidomide (len) 25mg PO d1-21, dexamethasone (dex) 40mg PO d1-4,8-9,15-16). Patients were randomised to this up-front quadruplet or the sequential strategy of CRD (cyclo 500mg PO d1,8, len 25mg PO d1-21 PO daily, dex 40mg PO d1-4,8-9,15-16) or nothing and those with SD/PD all received sequential CVD (cyclo 500mg PO d1,8,15 thalidomide 100-200mg PO daily, dex 40mg PO d1-4,12-15) given to max. response. Patients with VGPR/CR proceeded straight to ASCT, those with PR/MR were randomised to sequential CVD (cyclo 500mg d1,8,15, bortezomib 1.3mg/m2 IV/SC d1,4,8,11, dex 20mg PO d1,2,4,5,8,9,11,12) or nothing and those with SD/PO all received sequential CVD. At day 100 post ASCT there was a maintenance randomisation of CRD (cyclo 500mg PO d1,8, len 25mg PO daily, dex 40mg PO daily) or ASCT alone. All patients have completed induction therapy. The trial has now closed to recruitment and all patients have completed induction therapy. This analysis compares responses and toxicity of the different regimens.

Table 1.

<table>
<thead>
<tr>
<th>Table</th>
<th>Treatment exposure and safety data.</th>
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<tbody>
<tr>
<td>Response at end of first induction therapy</td>
<td>CRD: 4.5%, KCRD: 8.1%;</td>
</tr>
<tr>
<td>CRD</td>
<td></td>
</tr>
<tr>
<td>KCRD</td>
<td></td>
</tr>
<tr>
<td>CRD: maximal response</td>
<td></td>
</tr>
<tr>
<td>CRD: 9.6%</td>
<td></td>
</tr>
<tr>
<td>KCRD 10%</td>
<td></td>
</tr>
<tr>
<td>KCRD: 10%</td>
<td></td>
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</table>
| Grade ≥2 neurotoxicity was greater with the thalidomide-containing regimen (Sensory neuropathy CRD: 9.5%, CRD: 3.4%, KCRD: 2.3%). There was no statistically significant difference in rates of investigator reported, all-grade, thromboembolic events between regimens (CRD: 11.8%, CRD: 11%, KCRD 14.7%). Response to initial induction and following ASCT is shown in Table 1 indicating deeper responses with the quadruplet compared to triplets both at the end of first induction regimen (p<0.0001) and, importantly, post-ASCT (p<0.0001). These differences were observed despite the use of randomised pre-transplant consolidation for suboptimal responders to triplet immunomodulatory therapy.

Summary/Conclusions: Induction therapy with KCRD, an outpatient delivered quadruplet regimen, was associated with deeper responses than immunomodulatory triplet therapy (CRD/CTD) and was well tolerated. Deeper responses persisted after ASCT, with an impressive response rate ≥VGPR of 92% with KCRD.

S408

DEEP AND DURABLE RESPONSES WITH WEEKLY IXAZOMIB, LENALIDOMIDE AND DEXAMETHASONE IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA: LONG-TERM FOLLOW-UP OF PATIENTS WHO DID NOT UNDERGO SCT

S. Kumar1, J. Berdeja2, R. Niesvizky3, S. Lonial4, J. Laubach5, M. Hamadani6, A.K. Stewarte7, P. Harr8, V. Roy9, R. Vesco10,11, J. Kaufman11, D. Berg12, E. Liao12, V. Rajkumar1, P. Richardson5

1Mayo Clinic, Rochester, 2Sarah Cannon Research Institute, Nashville, 3Myeloma Center, Well Cornell Medical College, New York Presbyterian Hospital, New York, 4Department of Immunology and Medicinal Oncology, Winship Cancer Institute of Emory University, Atlanta, 5Dana-Farber Cancer Institute, Boston, 6West Virginia University, Mary Babb Randolph Cancer Center, Morgantown, 7Mayo Clinic College of Medicine, Scottsdale, 8Division of Hematology Oncology, Medical College of Wisconsin, Milwaukee, 9Mayo Clinic, Jacksonville, 10Cedars-Sinai Outpatient Cancer Center at the Samuel Oschin Comprehensive Cancer Institute, Los Angeles, 11Winship Cancer Institute of Emory University, Atlanta, 12Millennium Pharmaceuticals Inc., Cambridge, MA, USA, a wholly owned subsidiary of Takeda Pharmaceutical Company Limited, Cambridge, United States

Background: Triplet combinations that include a proteasome inhibitor (PI) have been proven superior to doublets in newly diagnosed multiple myeloma (NDMM) (San Miguel et al, N Engl J Med 2008, Durie et al, Lancet 2017). The all-oral combination of the novel PI ixazomib plus lenalidomide-dexamethasone (IRd) was evaluated as an induction regimen in NDMM patients, followed by single-agent ixazomib maintenance.

Aims: Here we report updated efficacy and long-term safety data for patients who did not withdraw from the study in order to receive stem cell transplantation (SCT).

Table 1.

<table>
<thead>
<tr>
<th>Table</th>
<th>Treatment exposure and safety data.</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients who did not undergo SCT</td>
<td>CRD: 4.5%, KCRD: 8.1%;</td>
</tr>
<tr>
<td>CRD</td>
<td></td>
</tr>
<tr>
<td>KCRD</td>
<td></td>
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<tr>
<td>CRD: maximal response</td>
<td></td>
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<tr>
<td>CRD: 9.6%</td>
<td></td>
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<tr>
<td>KCRD 10%</td>
<td></td>
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<tr>
<td>KCRD: 10%</td>
<td></td>
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</table>
| Grade ≥2 neurotoxicity was greater with the thalidomide-containing regimen (Sensory neuropathy CRD: 9.5%, CRD: 3.4%, KCRD: 2.3%). There was no statistically significant difference in rates of investigator reported, all-grade, thromboembolic events between regimens (CRD: 11.8%, CRD: 11%, KCRD 14.7%). Response to initial induction and following ASCT is shown in Table 1 indicating deeper responses with the quadruplet compared to triplets both at the end of first induction regimen (p<0.0001) and, importantly, post-ASCT (p<0.0001). These differences were observed despite the use of randomised pre-transplant consolidation for suboptimal responders to triplet immunomodulatory therapy.

Summary/Conclusions: Induction therapy with KCRD, an outpatient delivered quadruplet regimen, was associated with deeper responses than immunomodulatory triplet therapy (CRD/CTD) and was well tolerated. Deeper responses persisted after ASCT, with an impressive response rate ≥VGPR of 92% with KCRD.
Methods: In this phase 1/2 study (NCT01217957), patients with NDMM received weekly oral ixazomib (1.83-3.65mg/m²; days 1, 8, 15) and dexamethasone (40mg, days 1, 8, 15, 22) for up to 12-28-day induction cycles, followed by maintenance therapy with weekly single-agent ixazomib, at the last tolerated dose given during induction, until disease progression or toxicity.

Results: Of the 65 enrolled patients, 42 continued on study treatment without early withdrawal for SCT; the long-term follow-up of these 42 patients is reported here. Baseline patient characteristics included: median age, 68 years (range 34-86); ISS stage I/II/III in 40%/43%/17%. As of October 18, 2016, with median follow-up of 56 months, the confirmed overall response rate (ORR; partial response [PR] in, 80%; complete plus very good partial response (CR+VGPR) rate was 63%, and CR rate was 32%. Median time to first response was rapid (0.95 months), while median time to CR was 5.6 months. Median progression-free survival (PFS) in these patients not receiving SCT was 25.3 months. Median overall survival (OS) has not been reached at a median follow-up of 3 years. Minimal residual disease (MRD) was 87%. Safety findings are summarized in the Table: 74% of patients had grade 3 treatment-related adverse events (AEs), and 26% of the patients had treatment-related serious AEs. Among treatment-related AEs of interest, grade 3 rash and peripheral neuropathy were infrequent. There was one treatment-related death due to respiratory syncytial virus pneumonia. After completing 12 cycles of induction therapy with IRd, 25 patients went on to receive maintenance single-agent ixazomib. In these 25 patients, at the end of the induction period ORR was 100%, including 44%-100% and 32% and 0% of patients who received maintenance therapy was 24 months. The occurrence of the most common treatment-related grade ≥3 AEs (neutropenia, thrombocytopenia, and fatigue) was confined almost exclusively to the induction period. During the maintenance period no patients reported onset of grade ≥3 peripheral neuropathy or rash.

Summary/Conclusions: In patients with NDMM, weekly ixazomib plus Rd, followed by single-agent ixazomib maintenance, was highly active, resulting in deep and durable responses, long PFS, and a high 3-year OS estimate. IRd followed by single-agent ixazomib maintenance also showed an acceptable safety profile, with less toxicity reported during the maintenance (single-agent ixazomib) vs induction (IRd) periods, with no evidence of cumulative toxicities.

S409

DEPTH OF RESPONSE AS SURROGATE MARKER FOR PROGRESSION-FREE AND OVERALL SURVIVAL IN ELDERLY NEWLY DIAGNOSED MYELOMA PATIENTS TREATED WITH VMP AND RD: GEM2010MA0065


Background: Bortezomib plus melphalan and prednisone (VMP) and lenalidomide (25mg, days 1-21) and dexamethasone (40mg, days 1, 8, 15, and 22) for up to twelve 28-day induction cycles, followed by maintenance therapy with weekly single-agent ixazomib, at the last tolerated dose given during induction, until disease progression or toxicity.

Methods: Of the 65 enrolled patients, 42 continued on study treatment without early withdrawal for SCT; the long-term follow-up of these 42 patients is reported here. Baseline patient characteristics included: median age, 68 years (range 34-86); ISS stage I/II/III in 40%/43%/17%. As of October 18, 2016, with median follow-up of 56 months, the confirmed overall response rate (ORR; partial response [PR] in, 80%; complete plus very good partial response (CR+VGPR) rate was 63%, and CR rate was 32%. Median time to first response was rapid (0.95 months), while median time to CR was 5.6 months. Median progression-free survival (PFS) in these patients not receiving SCT was 25.3 months. Median overall survival (OS) has not been reached at a median follow-up of 3 years. Minimal residual disease (MRD) was 87%. Safety findings are summarized in the Table: 74% of patients had grade 3 treatment-related adverse events (AEs), and 26% of the patients had treatment-related serious AEs. Among treatment-related AEs of interest, grade 3 rash and peripheral neuropathy were infrequent. There was one treatment-related death due to respiratory syncytial virus pneumonia. After completing 12 cycles of induction therapy with IRd, 25 patients went on to receive maintenance single-agent ixazomib. In these 25 patients, at the end of the induction period ORR was 100%, including 44%-100% and 32% and 0% of patients who received maintenance therapy was 24 months. The occurrence of the most common treatment-related grade ≥3 AEs (neutropenia, thrombocytopenia, and fatigue) was confined almost exclusively to the induction period. During the maintenance period no patients reported onset of grade ≥3 peripheral neuropathy or rash.

Summary/Conclusions: In patients with NDMM, weekly ixazomib plus Rd, followed by single-agent ixazomib maintenance, was highly active, resulting in deep and durable responses, long PFS, and a high 3-year OS estimate. IRd followed by single-agent ixazomib maintenance also showed an acceptable safety profile, with less toxicity reported during the maintenance (single-agent ixazomib) vs induction (IRd) periods, with no evidence of cumulative toxicities.

S410

CARFILZOMIB-LENALIDOMIDE-DEXAMETHASONE VS CARFILZOMIB-CYCLOPHOSPHAMIDE-DEXAMETHASONE INDUCTION: PLANNED INTERIM ANALYSIS OF THE RANDOMIZED FORTE TRIAL IN NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background: Previous phase I-II studies showed that Carfilzomib-Lenalidomide-Dexamethasone (KRd) and Carfilzomib-Cyclophosphamide-Dexamethasone (KCD) combinations are safe and effective in patients with newly diagnosed multiple myeloma (NDMM) (Jaukowiaki Blood 2012, Bringhen Blood 2013). The FORTE trial compared KCD vs KRd for 18 cycles in a sequential alternating scheme. After a median of 27 months, both regimens (sequential and alternating) showed similar efficacy with an acceptable toxicity profile.

Aims: To consolidate data, we have updated the outcome with long follow-up (median of 51 months), evaluating the role of Complete Response and Minimal Residual Disease (MRD) in the analysis of treatment response.

Methods: 242 pts were randomized to receive 9 cycles of VMP followed by 9 cycles of RD or the same regimens in an alternating approach (one cycle of VMP alternating with one Rd, up to 18 cycles. VMP included iv administration of weekly bortezomib (except in the first cycle that was given twice weekly) at 1.3mg/m2, lenalidomide (25mg, days 1-21) and dexamethasone (40mg, days 1, 8, 15, and 22) for up to twenty-eight 28-day induction cycles, followed by maintenance therapy with weekly single-agent ixazomib, at the last tolerated dose given during induction, until disease progression or toxicity.

Results: In 281 patients were evaluated (94 assigned to KCD treatment and 187 to KRd treatment). The most frequent grade 3-4 adverse events (AEs) and serious AEs (SAEs) in both arms were hematological (mainly neutropenia and infections (mainly pneumonia/fever); increased AST/ALT/GGT (mainly reversible) and dermatological (rash) AEs were more frequent among KRd patients, cardiac AEs were 3% in KCD vs 6% in KRd (including atrial fibrillation [1%] and ischemic heart disease [1%]) vs 1% with KCD (atrial fibrillation). Death occurred in 1 patient in the KCD group (infection not treatment-related) and 3 patients in the KRd group (2 cardiac arrest [1 not treatment-related], 1 infection not treatment-related). In the KCD vs KRd arms, 96% vs 95% (P=0.44) of pts mobilized stem cells (median number of PBS collected: 9 vs 6x10^6CD34/kg with KCD vs KRd). Plerixafor was required in 10% vs 24% (P=0.01), respectively. At least a very good partial response (VGPR) was reported in 61% of patients receiving KCD vs 74% receiving KRd (P=0.05).
Table 1.

<table>
<thead>
<tr>
<th>Grade 3-4 AEs/SAEs</th>
<th>KCd</th>
<th>KRd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematological</td>
<td>13%</td>
<td>9%</td>
</tr>
<tr>
<td>Cardiac</td>
<td>1%</td>
<td>2%</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0%</td>
<td>1%</td>
</tr>
<tr>
<td>Thromboembolism</td>
<td>0%</td>
<td>1%</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>0%</td>
<td>3%</td>
</tr>
<tr>
<td>AST/ALT/GGT increase</td>
<td>6%</td>
<td>9%</td>
</tr>
<tr>
<td>Dermatological</td>
<td>0%</td>
<td>6%</td>
</tr>
<tr>
<td>Infections</td>
<td>0%</td>
<td>9%</td>
</tr>
<tr>
<td>Acute Kidney Injury</td>
<td>&lt;0.5</td>
<td></td>
</tr>
</tbody>
</table>

Summary/Conclusions: Safety profile was acceptable; more patients required plerixafor in the KRd arm. Rate of VGPR was higher with KRd. Updated data on a higher number of patients will be presented at the meeting. The trial is registered at Clinicaltrials.gov: NCT02203643

HOVON 104; FINAL RESULTS FROM A MULTICENTER, PROSPECTIVE PHASE II STUDY OF BORTEZOMIB BASED INDUCTION TREATMENT FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH DE NOVO ALAMYLOIDOSIS

M. Minnema1,*, K. Nasserinejad2, B. Hazenberg3, U. Hegenbart4, L. Noens5, P. Ypma6, S. Zweegman7, L. Tick8, A. Broijl9, H. Koene10, G. Bos11, N. Thuss2, P. Sonneveld8, S. Schonland1

1Haematology, UMC UTRECHT, Utrecht, 2HOVON data center, ErasmusMC, Rotterdam, 3Rheumatology, UMCG, Groningen, Netherlands, 4Amyloidosis Center, University of Heidelberg, Heidelberg, Germany, 5Haematology, UZ Gent, Gent, Belgium, 6Internal Medicine, HAGA hospitals, the Hague, 7Haematology, VU medical center, Amsterdam, 8Internal Medicine, Maxima Medical Center, Eindhoven, 9Haematology, ErasmusMC, Rotterdam, 10Internal Medicine, st Antonius Hospital, Nieuwegein, 11Haematology, University Hospital Maastricht, Maastricht, Netherlands

Background: Bortezomib (B) has been reported to be very effective in AL amyloidosis with overall response rates (ORR) varying between 50-80%. However, there are no prospective data from multicenter studies on B treatment in de novo patients. We investigated the efficacy and safety of B-Dexamethasone (BD) induction treatment followed by HDM+SCT in de novo AL amyloidosis patients.

Aims: The primary aim was to improve the hematological CR rate at 6 months after SCT on intention to treat analysis from 30 to 50%. Secondary aims were OS, PFS, hematological response rate after BD treatment, organ responses, safety and prognostic factors for survival.

Methods: Patients with biopsy proven AL amyloidosis, aged between 18-70 years, with detectable M-protein and/or level of involved FLC >50mg/L, WHO performance status 0-2, NYHA stage 1-2 and ejection fraction >45% were included. Major exclusion criteria were symptomatic orthostatic hypotension, NT proBNP level >5000 pg/ml, Troponin T > 0.06 ug/l, Bilirubin >2x ULN, CTCAE grade peripheral sensory neuropathy > grade 2 or > grade 1 with pain. Inclusion and exclusion criteria were installed both at entry and before stem cell mobilization (SCM). B was given subcutaneously 1.3mg/m² twice a week in a 21-day cycle, D 20mg orally on each B day and the following day. HDM dosage was 200mg/m². Hematological responses were defined according to consensus criteria with the addition of very good partial response (VGPR), defined as the difference between involved and uninvolved FLC<40mg/L. Cardiac, renal and liver response and progression criteria were defined according to consensus criteria with addition of NT proBNP.

Results: Median age was 59 years (range 26-70) and 60% were male. NYHA stage was 1 in 56% and 2 in 42% of patients. Mayo cardiac risk score was 1 (30%), II (36%), III (34%). Organ involvement was 82% renal, 66% heart, 28% liver, 14% neurological, 8% gastrointestinal and 38% of patients had 3 or more organs involved. Bone marrow plasmacells were >10% in 28% of patients. The median FU for patients alive is 24 (10-55) months. Twelve of 50 (24%) patients could not proceed to SCM. Four patients due to B related toxicity, 3 patients did not fulfill criteria to proceed, 2 patients died (both amyloidosis related) and 3 miscellaneous. Of these 38 patients, 3 went subsequently off protocol because of eligibility for HDM. Thirty-five out of 50 patients (70%) received HDM + SCT, one patient died of a cardiac arrest after the SCT procedure. The ORR after induction was 80%, ≥VGPR in 54% and CR in 6% of patients. The ORR in the 35 patients at 6 months after SCT was 80%, ≥VGPR in 51% and CR in 43% of patients. On intention to treat analysis the CR rate at 6 months after SCT was 30%. Organ responses at 6 months after SCT were 16/29 renal, 2/8 liver and 13/23 heart. No baseline characteristics were identified to be predictive for OS or PFS. BD doses were reduced and delayed after 2 cycles in almost half of patients, mostly because of neurotoxicity. Sensory neuropathy grade 2 or higher was seen in 36% of patients and autonomic neuropathy, mostly dizziness and collapse, in 22%.

Summary/Conclusions: This final analysis demonstrates that the primary aim of improving CR rate at 6 months after SCT from 30 to 50% was not met. This was mainly caused by the high dropout rate before SCT. This may be due to patient selection, but we also demonstrate that BD, given twice weekly sc, despite good efficacy, cannot prevent early amyloidosis related toxicity and can induce grade 2 or higher neurotoxicity.

Trial registration www.trialregister.nl (NTR 3220), EudraCT 2010-021445-42, supported by the Dutch Cancer Society (UU 2010-4884) and by an unrestricted grant from Janssen-Cilag.
**Hodgkin and indolent lymphoma - Clinical**

**S412**

**NIVOLUMAB FOR RELAPSED/REFRACTORY CLASSICAL HODGKIN LYMPHOMA AFTER AUTOLOGOUS TRANSPLANT: FULL RESULTS AFTER EXTENDED FOLLOW-UP OF THE MULTICOHORT MULTICENTER PHASE 2 CHECKMATE 205 TRIAL.**

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**Background:** Nivolumab, a fully human IgG4 monoclonal antibody targeting programmed death-1, is an immune checkpoint inhibitor that augments T-cell activation and antitumor responses. Nivolumab is indicated for pts with relapsed/refractory (RR) classical Hodgkin lymphoma (cHL) following autologous stem cell transplantation (ASCT) and brentuximab vedotin (BV) treatment. The multicohort phase 2 CheckMate 205 trial (NCT02181738) enrolled pts with RR cHL after ASCT. Initial analyses revealed high objective response rates (ORR), encouraging duration of response (DOR) and an acceptable safety profile (Younes A et al, Lancet Oncol 2016). Durable responses to therapy are valuable in pts with progressive disease after failure of ASCT due to their limited treatment options.

**Aims:** To report extended follow-up data for all pts with RR cHL after failure of ASCT in CheckMate 205.

**Methods:** This is a single-arm multicenter trial enrolled pts (age ≥18 y) with RR cHL after ASCT into 1 of 3 independent cohorts (Cohort A: BV-naïve; Cohort B: BV only after ASCT; Cohort C: BV before and/or after ASCT). All pts received nivolumab 3mg/kg every 2 wk until disease progression or unacceptable toxicity. Pts in Cohort C with a persistent complete response (CR) for 1 y were to discontinue nivolumab and be treated with either consolidation radiotherapy (Rxt) on the sites of initial large nodal mass (LNM: diameter >5cm) or no further treatment (NFT).

**Results:** In total, 243 pts were treated: 63 in Cohort A (BV-naïve), 80 in Cohort B (BV after ASCT), and 100 in Cohort C (BV before [n=53], after [n=58], or both after [n=29]). Median (range) age was 34 (18-72) y and 77% of pts had advanced (stage III+) disease at study entry. BV-naïve pts had fewer prior lines of therapy (median of 2 vs ≥4 with prior BV). At Dec 2016 database lock, median (min, max) follow-up was 19 (1, 25), 23 (2, 27) and 16 (1, 20) mo in Cohorts A, B, and C, respectively. Overall, 40% of pts were still on treatment; the most common reason for discontinuation was disease progression (26%). ORR was 65% in Cohort A, 68% in Cohort B, and 73% in Cohort C, with 29%, 13%, and 12% CR, respectively. Median (95% CI) DOR was 20 (13, 20), 16 (8, 20), and 15 (9, 17) mo in Cohorts A, B, and C, respectively. DOR for patients with CR was 20 months for BV-naïve patients (Cohort A) and ≥15 mo for BV-treated patients (Cohorts B and C); DOR for patients with partial response (PR) was 17 and ≥11 months, respectively. PFS by cohort is shown (Figure 1). Prolonged median PFS was seen for patients with CR (≥17 mo in each cohort), PR (≥15 mo in each cohort), and stable disease (≥9 mo in each cohort). Median OS was not reached in any cohort. The most common drug-related AEs were grade 3-4 drug-related AEs in ≥3% of pts were lipase increases (5%), alanine aminotransferase increases (3%), and neutropenia (3%). The most common drug-related serious AEs were irAEs (2%) and pneumonitis (1%). To facilitate translation to practice, efficacy results by sequencing of prior BV treatment will be presented.

**Summary/Conclusion:** With extended follow-up, high and durable rates of CR and PR to nivolumab therapy were observed in pts with RR cHL after ASCT, irrespective of BV treatment history.

**Study funding:** BMS; medical writing support: M Thomas (Caudex), funded by BMS.

**S413**

**EARY CHEMOTHERAPY INTENSIFICATION WITH ESCALATED BEACOPP IN ADVANCED-STAGE HODGKIN LYMPHOMA WITH A POSITIVE INTERIM PET-CT AFTER 2 PET-CT CYCLES: LONG-TERM RESULTS OF THE GITL/FIL HD 8607 TRIAL.**


**Background:** Interim 2-[18F]fluoro-2-deoxy-D-glucose Positron Emission Tomography (FDG-PET) performed after 2 chemotherapy cycles (PET2) is the most powerful predictor of treatment outcome in ABVD-treated, advanced-stage classical Hodgkin lymphoma (cHL). Preliminary reports showed that adapting treatment to PET2 result could increase the efficacy of standard ABVD.

**Aims:** To confirm in a prospective setting the favorable prognosis of advanced stage PET2 negative patients treated with ABVD, as well as the safety and efficacy of escalated BEACOPP given to PET2 positive patients.

**Methods:** We conducted a prospective clinical trial (HD0607 ClinicalTRial.gov identifier 007796513), in which advanced-stage (IIB-IVB) cHL patients were treated with 2 ABVD courses, and PET2 performed afterwards. The latter was blindly and independently reviewed by a panel of nuclear medicine experts, using the Deauville 5-point scale (5-PS). PET2+ patients (5-PS 4-5) were randomized to either BEACOPP escalated (Be+) or plus BEACOPP baseline (Be) (Be+ vs or Be+Bb (4+4) and Rituximab (R). PET2- (5-PS 1 to 3) patients continued ABVD treatment with 4 more cycles and, upon CR achievement, randomized to either consolidation radiotherapy (Rtx) on the sites of initial large nodal mass (LNM: diameter >5cm) or no further treatment (NFT). Aims: To report results of a randomized phase 2 CHEMAT trial (HD0607 ClinicalTRial.gov identifier 007796513), in which advanced-stage (IIB-IVB) cHL patients were consecutively enrolled in 24 Italian and 1 Israeli centers. The median age was 31 years (14-60); 35% had stage IIB, 32% stage III and 32% stage IV. The International Prognostic Score (IPS) was 0-1 in 36.6%, 2-3 in 51%, >3 in 12.5%.
After 3rd or higher relapse of classical Hodgkin lymphoma (cHL) are sparse. Therefore the additional benefit of new agents, which were cumulatively investigated after several relapses of cHL, is difficult to estimate.

**Aims:** The aim of this study was to define and describe a historical control group in European patients from the German Hodgkin Study Group (GHSG) data for comparison of safety and efficacy of novel therapeutic agents.

**Methods:** Cases with at least three consecutive chemotherapy-related events or progressive refractory or relapsed disease, were identified in the GHSG database. Detailed information was added from case report forms and physician’s letters. Overall survival (OS) was the main and progression free survival (PFS), response to therapy, adverse events, disease and treatment characteristics as secondary endpoints.

**Results:** Among 12,584 HL patients in the GHSG first-line trials HD7 to HD15 and 449 HL patients in the trials HDR1 and HDR2 a total of 69 cHL patients with ≥3 tumor events were identified. The dates of occurrence of 3rd relapse ranged between 15th of January 1993 and 21st of June 2013. The sample consisted of 51 male (74%) and 18 female (26%) patients. At time of 3rd relapse the age of the patients ranged from 20 to 79 years (mean 39.2 years, standard deviation (SD) 14.0 years) and the majority of patients presented with stage III or IV disease (67%). Time from end of 3rd-line treatment to 3rd relapse was ≤3 months (i.e. GHSG definition of refractory disease) in 15 cases (22%), ≤12 months (early relapse) in 19 cases (28%) and >12 months (late relapse) in 35 cases (51%). All 69 patients were pretreated with chemotherapy, 35 (50.7%) with BEACOPP, 30 (43.5%) with ABVD and no BEACOPP, and 32 (46.6%) with another type of chemotherapy. The number of prior chemotherapies ranged from one to three (median 3). Pretreatment with radiotherapy was observed in 57 (82.6%) patients, with salvage chemotherapy aimed to induce a remission prior to a stem-cell transplantation (SCT) in 58 (84.1%), and with high dose chemotherapy followed by autologous SCT in 50 (72.5%) patients. Four patients (5.8%) had received allogeneic SCT as 3rd-line treatment. None of the patients had received brentuximab vedotin or anti-PD1 antibodies before 3rd relapse. With a median observation time of 63.3 months for OS after 3rd relapse, 45 patients (65.2%) had died and 60 (87.0%) had another PFS event. Twelve months after the 3rd relapse OS was 73.2% (95%-CI 62.6% to 83.8%) and PFS 50.8% (95%-CI 38.9% to 62.8%, Table 1).

### Table 1.

<table>
<thead>
<tr>
<th>Progression Free Survival (PFS)</th>
<th>Overall Survival (OS)</th>
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<tr>
<td>12 months</td>
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<tr>
<td><strong>events</strong></td>
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<td>53.4%</td>
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</table>

Summary/Conclusions: Patients with a 3rd relapse or progression of cHL have a dismal, mostly palliative prognosis due to frequent tumor progression. Within one year half of the patients have a PFS event and one fourth die.

**S414**

**DISEASE CHARACTERISTICS AND SURVIVAL AFTER 3RD RECURRENT OF CLASSICAL HODGKIN LYMPHOMA: AN ANALYSIS OF THE GERMAN HODGKIN STUDY GROUP**


**Background:** Data on disease presentation, therapeutic options and survival after 3rd or higher relapse of classical Hodgkin lymphoma (cHL) are sparse. Therefore the additional benefit of new agents, which were cumulatively investigated after several relapses of cHL, is difficult to estimate.

**Aims:** The aim of this study was to define and describe a historical control group in European patients from the German Hodgkin Study Group (GHSG) data for comparison of safety and efficacy of novel therapeutic agents.

**Methods:** Cases with at least three consecutive chemotherapy-related events or progressive refractory or relapsed disease, were identified in the GHSG database. Detailed information was added from case report forms and physician’s letters. Overall survival (OS) was the main and progression free survival (PFS), response to therapy, adverse events, disease and treatment characteristics as secondary endpoints.

**Results:** Among 12,584 HL patients in the GHSG first-line trials HD7 to HD15 and 449 HL patients in the trials HDR1 and HDR2 a total of 69 cHL patients with ≥3 tumor events were identified. The dates of occurrence of 3rd relapse ranged between 15th of January 1993 and 21st of June 2013. The sample consisted of 51 male (74%) and 18 female (26%) patients. At time of 3rd relapse the age of the patients ranged from 20 to 79 years (mean 39.2 years, standard deviation (SD) 14.0 years) and the majority of patients presented with stage III or IV disease (67%). Time from end of 3rd-line treatment to 3rd relapse was ≤3 months (i.e. GHSG definition of refractory disease) in 15 cases (22%), ≤12 months (early relapse) in 19 cases (28%) and >12 months (late relapse) in 35 cases (51%). All 69 patients were pretreated with chemotherapy, 35 (50.7%) with BEACOPP, 30 (43.5%) with ABVD and no BEACOPP, and 32 (46.6%) with another type of chemotherapy. The number of prior chemotherapies ranged from one to three (median 3). Pretreatment with radiotherapy was observed in 57 (82.6%) patients, with salvage chemotherapy aimed to induce a remission prior to a stem-cell transplantation (SCT) in 58 (84.1%), and with high dose chemotherapy followed by autologous SCT in 50 (72.5%) patients. Four patients (5.8%) had received allogeneic SCT as 3rd-line treatment. None of the patients had received brentuximab vedotin or anti-PD1 antibodies before 3rd relapse. With a median observation time of 63.3 months for OS after 3rd relapse, 45 patients (65.2%) had died and 60 (87.0%) had another PFS event. Twelve months after the 3rd relapse OS was 73.2% (95%-CI 62.6% to 83.8%) and PFS 50.8% (95%-CI 38.9% to 62.8%, Table 1).

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<td>53.4%</td>
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**Aims:** The aim of the current study was to revise the current IPSSWM by using a large dataset of symptomatic WM patients treated with different types of primary therapy that included rituximab and other new agents.

**Methods:** The analysis included 492 patients from the prospectively maintained database of the Greek Myeloma Study Group with a median follow up of 10 years. All patients fulfilled criteria for diagnosis and for treatment initiation according to Consensus Recommendations.

**Results:** In univariate analysis factors such as age, beta-2 microglobulin, serum albumin and LDH were all associated with poor outcome. The IPSSWM includes age and b2 microglobulin but not serum albumin, or LDH, while the presence of very high IgM (>7 gr/dl) was quite rare and of limited prognostic value. The presence of anemia <11.5 gr/dl was common across all subgroups while low platelet counts <100×10^9/L was found in relatively few patients and had no prognostic significance. Based on ROC analysis for early death (within 3 years), serum albumin <3.5 gr/dl and b2microglobulin >4 mg/L were the two most important prognostic factors of early WM-related death. Age >65 years was associated with increased risk of death, however, age >75 years conferred additional risk (double hazard of death compared to those 65-75 years and fourfold compared to patients <65 years). Thus, we formulated a score in which high b2 microglobulin, elevated LDH and low serum albumin are scored with 1 point each, age 66-75 years is scored with 1 point but age >75 years is scored with 2. As a result, patients with scores 0, 1, 2, 3 or 4-5 had 3-year WM-related death rate of 3%, 7%, 14%, 19% and 48% (chi-square: 80.7, p<0.001). Regarding overall survival, 10-year survival rate was 85%, 59%, 39%, 28% and 12% (p=0.001) (Figure 1). Because age is a major determinant of disposition we also evaluated this staging system in patients >65 years and retained it prognostic significance. Compared to IPSSWM, this new staging system outperformed ISSWM: c-statistics, a measure of performance of a prognostic tool, was 0.652 vs 0.711 (95% CI 0.659-0.763) vs 0.711 (95% CI 0.659-0.763) for the new staging system.

**Summary/Conclusions:** A revised staging system, based on b2 microglobulin, elevated LDH, low serum albumin and age identifies groups with very different outcomes among patients with symptomatic WM treated with contemporary regimens and may outperform IPSSWM.

**Figure 1.**

**Table 1.**

<table>
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<tr>
<td>10 years OS</td>
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<tr>
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**Table 2.**

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</table>

**S416**

**SPLENIC MARGINAL ZONE LYMPHOMA (SMZL) TREATED WITH RITUXIMAB (R) MONOTHERAPY: A LONG TERM FOLLOW-UP STUDY ON 104 PATIENTS**

C. Kalpadakis1,2, G. Pangalis2, T. Vassilakopoulos1, S. Sachanas2, M. Moschogiannis3, P. Tsirkidis3, X. Yiakoumis3, D. Rentogianni4, F. Kontopidou5, S. Kyriakaki1, M. Psyllaki1, A. Dimitrakopoulou6, E. Koulieris2, M.-C. Kyrtsonis7, M. Siakantaris8, P. Korkolopoulou9, T. Tzenou3, H. Papadaki1, M. Angelopolou3, 1Department of Haematology, Athens Medical Center-Psychikon Branch, 2Department of Haematology, University of Athens, Laikon General Hospital, 3Department of Anatomic Pathology, Evangelismos General Hospital, University of Athens, 42nd Department of Internal Medicine, University of Athens, 5Immunology Laboratory, Laikon General Hospital, 61st Department of Propedeutics, University of Athens, Laikon General Hospital, 7First Department of Internal Medicine, 8Department of Pathology, University of Athens, Athens, Greece

**Background:** Rituximab monotherapy has been used successfully in the treatment of SMZL and it can replace splenectomy, at least in 1st line.

**Aims:** To present our data on the outcome of R monotherapy treated pts after a long term follow-up.

**Methods:** The diagnosis of SMZL was based on the WHO criteria. Criteria for treatment initiation included: bulky/symptomatic splenomegaly, cytopenias or presence of B-symptoms. All pts received 6 weekly cycles of R as 1st line therapy at a dose of 375mg/m² (induction phase). None of the pts had been splenectomised before R treatment. Maintenance with R at a dose of 375mg/m² every 2 months for 1-2 years was given according to physician’s discretion. Response assessment was based on the SLG consensus criteria. Survival curves were estimated using the Kaplan Meier method and compared by log-rank test.

**Results:** 104 pts with SMZL were included. 45% were males with a median age of 66 y (41-91). At diagnosis all pts had bone marrow infiltration with a median % of infiltration of 40 (% 10-85). Anemia and thrombocytopenia were present in 30% and 19%, respectively. 40% had absolute lymphocytosis. LDH was elevated in 43%. According to the SLG prognostic system, 39% were classified in group A, 56% in group B and 5% in group C. The median time from diagnosis to treatment initiation was 2 months (0-203). 71 pts received R maintenance. The overall response rate 2 months after the end of induction treatment was 93% (CR, CRu and PR in 42%, 21% and 30%, respectively). Maintenance therapy improved the quality of response in 19 of them, 52 pts maintained their initial response and one relapsed during maintenance phase. The 5- and 10-year PFS, OS and CSS were 70%, 64%, 93% and 88%, 99% and 93%, respectively. Maintenance therapy was associated with better PFS (p=0.008). 22 pts relapsed (6 of them with histologic transformation to DLBCL), 11/22 were retreated with R and 9/11 responded. 8 deaths were recorded: 3 of them disease related. R therapy was well tolerated. Only one pt could not complete treatment due to intolerance.

**Summary/Conclusions:** The present study, includes a large number of pts with a long follow-up, confirms that R monotherapy is very effective in SMZL with minimal toxicity and is recommended as the treatment of choice for this disease.
YOUN DON'T KNOW JAK: A PROGRAMMED RIBOSOMAL FRAMESHIFTING DEFECT POTENTIATES THE TRANSFORMING ACTIVITY OF THE JAK2-V617F MUTATION

S. Sulima1, Y. Khan2, J. Briggs2, S. Vereecke1, J. Jones2, V. Advani2, J. Verbeeck1, K. De Keersmaecker1, J. Dinman2
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Background: The JAK-STAT pathway is a critical controller of cellular proliferation, differentiation, survival and apoptosis in response to external stimuli. Promiscuous activation of this pathway is an important driver in the pathogenesis of BCR-ABL-negative chronic myeloproliferative neoplasms. The JAK2-V617F allele is the most common and characterized mutation linked to this class of leukemia. The increased activation of JAK-STAT signaling in JAK2-V617F cells can be partially explained by increased JAK2 autophosphorylation. It is unclear how these effects are sufficient to fully account for the strong activation of the JAK-STAT pathway induced by JAK2-V617F. We recently described programmed -1 ribosomal frameshifting (-1 PRF) as a novel mechanism regulating the expression of ~10% of human genes, including cytokine receptors (Blewe AT el, Nature, 2014). In this process, cis-acting mRNA elements (-1 PRF signals, which consist of a slippery site followed by a pseudoknot) directly translating ribosomes to slip by one base in the 5’ direction, establishing a new reading frame. This directs ribosomes towards premature termination codons, resulting in destabilization of the -1 PRF signal-containing mRNA via nonsense-mediated mRNA decay (Figure 1). There is thus an inverse relationship between -1 PRF efficiency and mRNA stability.

Aims: To investigate whether the JAK2-V617F mutation, shown here to be located in the pseudoknot of a -1 PRF signal in the JAK2 mRNA, impacts disease progression through ablation of -1 PRF.

Methods: Computationally predicted -1 PRF signals were validated using dual luciferase reporters and proteomic analysis of -1 PRF fusion protein. -1 PRF results suggest a prominent role for -1 PRF in controlling JAK2 production. Computational predictions of -1 PRF sites can be validated using dual luciferase reporters and proteomic analysis of -1 PRF fusion protein. -1 PRF efficiency as well as mRNA abundance and decay were assayed in HEK293T and HeLa cells. Transformation assays were performed in HEK293T expressing Ba/F3 cells, in vivo experiments were performed in BALB/c mice.

Results: We demonstrate in human cell lines that the JAK2-V617F mutation structurally disrupts the -1 PRF signal in the JAK2 mRNA, leading to -2-fold lower rates of -1 PRF and increased abundance of the JAK2 mRNA and protein. The transforming potential of a series of mutants designed to manipulate -1 PRF independent of V617F was assayed in a Ba/F3 cell model. Silent protein coding changes in the pseudoknot of the -1 PRF signal at position V617F (V617Fm) or the slippery site (SSm), both of which reduced frameshifting, increased JAK2 expression and led to transforming activity, albeit less than V617F. Importantly, the V617F+SSm combination conferred an additive effect on cellular transformation. Ba/F3 cells expressing these JAK2 variants were also introduced into mice. Whereas mice injected with wild type JAK2 remained healthy, both V617F and SSm induced similar leukemia phenotypes as V617F and V617F+SSm, with a ~2-fold longer disease latency of 8-10 weeks. Increased JAK2 mRNA abundance in JAK2-V617F homozygous patients as well as the presence of three additional -1 PRF signals in the JAK2 mRNA further suggest a prominent role for -1 PRF in controlling JAK2 production. Conclusions: We demonstrate that the JAK2-V617F mutation diminishes -1 PRF on the JAK2 transcript, stabilizing the mRNA and increasing JAK2 expression, contributing to its transforming activity in vitro and disease onset in vivo. We suggest that -1 PRF normally provides a layer of control by limiting JAK2 translation. Defective -1 PRF synergizes with the transforming activity of the JAK2-V617F protein by causing its overexpression, explaining why this particular mutation causes such aggressive malignancies. In support of this, the combination of ruxolitinib and an HS-90 inhibitor, which reduce kinase activity and JAK2 expression respectively, leads to increased therapeutic efficacy in myeloproliferative neoplasms (Bhagvat N et al, Blood, 2014).
Aims: In this work, we aimed at investigating the role of RKIP in the development of CMML.

Methods: RKIP expression was measured by immunoblot and quantitative real-time PCR in 23 primary CMML patient samples as well as in CD34+ HSCs, B-lymphocytes, granulocytes and monocytes from four healthy donors. Sequence analysis of CMML samples was done with an Ion Torrent Next Generation Sequencing platform using an amplicon panel covering 39 genes recurrently mutated in myeloid neoplasms. Effects of RKIP on GM-CSF-induced myelomonocytic differentiation were studied in human CD34+ HSCs lentivirally transduced with RKIP shRNA, as well as in a genetic mouse model for RKIP deletion (RKIP<sup>-/-</sup>). Effects of RKIP on CMML development were initially studied in the same RKIP<sup>-/-</sup> model. Additionally, these mice were crossed with animals exhibiting a somatically inducible mutation in NRAS (RKIP<sup>-/-</sup>Cre-NRAS<sup>G12D</sup>) and the severity of CMML-MPD onset was studied at an age of six months.

Results: Loss of RKIP protein expression was observed in 6/23 (26%) CMML patient specimens and was associated with decreased mRNA levels as well (P<0.001). Patients with RKIP loss exhibited an increased percentage of myelomonocytic cells in the peripheral blood (56% vs 75%; P=0.0226). One or more mutations affecting the RAS signaling pathway were detected in all specimens with RKIP loss. In addition to the previously demonstrated induction of proliferation, we then aimed to delineate a role of RKIP loss in myeloid lineage commitment. When studying healthy donors, we observed that RKIP expression was high in HSCs and lymphoid cells, but significantly decreased in cells belonging to the myeloid lineage (monocytes, P=0.001 and granulocytes, P<0.001). In functional experiments, knockdown of RKIP increased the GM-CSF-induced myelomonocytic lineage commitment of both, human and murine HSCs (P<0.05 and P=0.0295, respectively). These results could be corroborated in vivo, as intraperitoneal injection of GM-CSF caused a significant increase of myelomonocytic cells in the intraperitoneal cavity (P=0.006), bone marrow (P=0.007) and peripheral blood (P=0.027) in RKIP<sup>-/-</sup> mice when compared to their wildtype littermates. In a final step, we evaluated the potential of RKIP loss to cause CMML-MPD in mice. While it proved to be insufficient to cause the disease as a single event in RKIP<sup>-/-</sup> mice, it aggravated the CMML-MPD phenotype in animals carrying an additional mutation in NRAS. In this case, RKIP deletion caused worsening of leukocytosis (P=0.036) and splenomegaly (P=0.035), which was associated with increased levels of myelomonocytic cells in the bone marrow (P=0.028), peripheral blood (P=0.002) and spleen (P=0.025).

Summary/Conclusions: RKIP loss is a frequent event in CMML and is associated with mutations affecting the RAS signaling cascade. Loss of RKIP is functionally involved in myelomonocytic lineage commitment of HSCs and aggravates CMML-MPD development in mice carrying an additional mutation in NRAS.
**Background:** The hematopoietic stem cell (HSC) compartment in mice encompasses a broad range of heterogeneous cell types including highly lineage-biased HSCs, such as platelet-biased HSCs (PIMID:23934107). Myeloproliferative neoplasms (MPNs) are a heterogeneous spectrum of clonal hematopoietic disorders, that includes essential thrombocytopenia (ET), a MPN subtype usually presenting with isolated thrombocytosis. Most ET patients carry a gain-of-function point mutation in JAK2 (JAK2V617F), with several other collaborating hits reported to co-occur with JAK2V617F at lower frequencies, including the previously described hJAK2V617F-associated transplantation defect in serial bone marrow (BM) transplantation assays, EZH2-KO fully rescued mice which became acutely unwell with an extreme thrombocytosis. Strikingly, hJAK2V617F mice showed increased platelet counts, including a subset of mice which became acutely unwell with an extreme thrombocytosis. Strikingly, in serial bone marrow (BM) transplantation assays, EZH2-KO fully rescued the previously described hJAK2V617F-associated transplantation defect (PIMID:20489053). EZH2-KO hJAK2V617F BM recipients showed long-term engraftment that was fully restricted to the platelet and myeloid lineages with a persistent thrombocytosis and absence of lymphoid reconstitution. RNA-sequencing revealed upregulation of several signaling pathways, including Hedgehog, and increased inflammation associated gene expression in EZH2-KO hJAK2V617F HSCs. Unexpectedly in this mouse model of thrombocytosis, phenotypic analysis of the HSC compartment in the BM showed that vwf-eGFP+ve HSCs were selectively lost (fold change [FC]=0.12 p=0.009), while vwf-eGFP–ve HSC numbers remained unaffected (FC=1.06 p=0.88) in EZH2-KO hJAK2V617F HSCs. Unexpectedly in this novel mouse model of MPN that carries a conditional knock-in of heterozygous human JAK2V617F (hJAK2V617F) and the conditional knock-out (KO) of EZH2 together with an inducible Mx1-Cre transgene. To analyse platelet-biased HSC subsets upon onset of the mutation(s), we also crossed the vwf-eGFP transgene, which is selectively expressed in platelet-biased HSCs.

**Results:** Compared to wild-type and single mutant mice, EZH2-KO hJAK2V617F mice showed increased platelet counts, including a subset of mice which became acutely unwell with an extreme thrombocytosis. Strikingly, in serial bone marrow (BM) transplantation assays, EZH2-KO fully rescued the previously described hJAK2V617F-associated transplantation defect (PIMID:20489053). EZH2-KO hJAK2V617F BM recipients showed long-term engraftment that was fully restricted to the platelet and myeloid lineages with a persistent thrombocytosis and absence of lymphoid reconstitution. RNA-sequencing revealed upregulation of several signaling pathways, including Hedgehog, and increased inflammation associated gene expression in EZH2-KO hJAK2V617F HSCs. Unexpectedly in this model of thrombocytosis, phenotypic analysis of the HSC compartment in the BM showed that vwf-eGFP+ve HSCs were selectively lost (fold change [FC]=0.12 p=0.009), while vwf-eGFP–ve HSC numbers remained unaffected (FC=1.06 p=0.88) in EZH2-KO hJAK2V617F mice. To assess a differential contribution of vwf-eGFP+ve HSCs vs vwf-eGFP–ve HSCs in the ability to propagate MPN, we sorted HSCs according to vwf-eGFP expression and transplanted them into recipient mice. Unlike their normal counterparts, which showed lymphoid-biased reconstitution, vwf-eGFP–ve HSCs from EZH2-KO hJAK2V617F mice primarily gave rise to platelets and myeloid cells. In contrast, vwf-eGFP+ve HSCs from EZH2-KO hJAK2V617F mice engrafted poorly without recapitulating the disease in recipients.

**Summary/Conclusions:** In this novel EzH2-KO hJAK2V617F mouse model, EzH2 loss collaborates to worsen thrombocytosis and rescue the HSC function defect in hJAK2V617F mice. We also observed a striking disruption of phenotypic and functional HSC heterogeneity in EzH2-KO hJAK2V617F mice with an unexpected and selective loss of vwf-eGFP+ve HSCs together with subversion of vwf-eGFP–ve HSCs towards platelet-myalid lineage commitment. This previously undescribed disruption of HSC heterogeneity in myeloid malignancy together with the clonal advantage conferred to HSCs by EZH2-KO helps to explain how this collaborating mutation might promote the development of more advanced MPN.

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**Clinical trials including treatment discontinuation in CML**

**S422**

**DASATINIB IN CHILDREN AND ADOLESCENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP) FROM A PHASE 2 TRIAL**


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**Background:** As safe and effective frontline treatment options for children and adolescents with CML are limited, and no approved therapies exist for patients (pts) resistant/intolerant to imatinib (IM), additional treatment options and alternative formulations are greatly needed for this younger population. Dasatinib (DAS) has proven efficacy in adults with newly diagnosed CML-CP, as well as those resistant/intolerant to IM (Cortes JCO 2016, Shah AJH 2016). Results of a phase 1 study confirmed its dosing and safety in pediatric pts (Zwaan JCO 2013); however, a larger prospective study is necessary to further support the use of DAS in pediatric pts with newly diagnosed or IM-resistant/intolerant CML-CP.

**Aims:** To determine whether DAS is safe and effective in pediatric pts with CML-CP newly diagnosed or resistant/intolerant to IM enrolled in a phase 2, open-label, nonrandomized prospective clinical trial (CA180-226/NC07077 7036).

**Table 1.**

<table>
<thead>
<tr>
<th>DAS</th>
<th>CML-CP (n=13)</th>
<th>Median daily dose, mg/kg</th>
<th>Median duration of treatment, months</th>
<th>Percentage of patients responding</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>55 (27-78)</td>
<td>50 (16-76)</td>
<td>7 (5-10)</td>
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Methods: Pts aged <18 years were recruited into 3 separate cohorts: (1) IM-resistant/intolerant CML-CP treated with DAS tablets 60mg/m² QD, (2) IM-resistant/intolerant CML-CP treated with DAS tablets 60mg/m² or DAS 72mg/m² powder for oral suspension (PFOS) QD for 1 year. PFOS dose was increased by 20% to match the exposure of the tablet in order to maintain efficacy based on the findings from a bioequivalence study in adults. Primary objectives were major cytogenetic response (MCyR) for CML-CP resistant/intolerant to IM and complete cytogenetic response (CCyR) for newly diagnosed CML-CP (MCyR >30% and CCyR >55% considered clinically relevant). Study cohorts were not designed to be comparative.

Results: From 145 pts enrolled, 130 were treated; 54% were aged ≥12-<18 years. Within the IM-resistant/intolerant group, 25 were resistant, 2 were intolerable, and 2 were undetermined. For pts with CML-CP (n=113), 48% of pts with IM-resistant/intolerant CML-CP and 73% with newly diagnosed CML-CP remained on treatment at the time of this analysis (table 1). Cumulative rate of MCyR was reached only as 3 months for IM-resistant/intolerant CML-CP, and a cumulative rate of CCyR >55% was reached as early as 6 months for newly diagnosed CML-CP (Table). Estimated progression-free survival (PFS) by 48 months was 78% for IM-resistant/intolerant CML-CP and 93% for newly diagnosed CML-CP (Table). Reasons for progression were loss of MCyR (n=3 IM-resistant/intolerant; n=4 newly diagnosed), loss of complete hematologic response (n=2 each), and development of CML-CP (n=2 IM-resistant/intolerant; n=1 newly diagnosed). One death was reported in the IM-resistant/intolerant CML-CP cohort 1 year after stopping DAS (gastrointestinal bleeding). Adverse events (AEs) were consistent with reports in DAS-treated adults, except no DAS-related hematologic events were observed. For pts with MCyR, McFarlane and others considered pulmonary arterial hypertension were reported here. Sensitivity in a newly diagnosed pt was the only DAS-related AE that led to discontinuation.

Summary/Conclusions: Results from the largest prospective and registration trial of pediatric pts with CML-CP demonstrate that DAS is a safe and effective treatment for pediatric CML-CP. Target responses to first- or second-line that DAS were met as early as 3 and 6 months, respectively, and deep responses were observed. Efficacy and safety of DAS in pediatric pts were similar to those observed in adults; however, unlike in adults, no cases of pleural/pericardial effusion were reported.
LEUKEMIA: INITIAL RESULTS FROM THE BFORE TRIAL

Survival. Outcome of CML is currently more determined by disease biology and demographics than by treatment optimization.

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Background: The optimum initial treatment of chronic myeloid leukemia (CML) is unknown.

Aims: CML-study IV was designed to confirm the International Randomized Study on Interferon (IFN) and STI571 (IRIS) and to explore whether treatment with imatinib (IM) at 400mg/day could be optimized.

Methods: From July 2002 to March 2012, 1551 newly diagnosed patients in chronic phase (CP) of CML were randomized into a 5-arm study. 1536 patients were evaluable, 400 for IM400mg, 430 for IM + IFN, 420 for IM800mg, 158 for IM + cytarabine and 128 for IM-after-IFN-failure. Recruitment to the latter two arms was stopped after a pilot-phase.

Results: After a median observation time of 9.5 years, 10-year overall survival (OS) of all patients was 82%, 10-year progression free survival 80% and, 10-year relative survival 92%. 10-year OS was 80% with IM400mg, 78% with IM800mg, 79% with IM + IFN, 79% with IM800mg, 84% with IM + cytarabine and 79% with IM after IFN (Figure 1). The differences were not significant in spite of faster response with IM800mg. In a multivariate analysis, risk group, comorbidities, major route chromosomal aberrations, smoking and type of treatment center (academic vs university) influenced survival, but not gender, transcript type or any form of treatment optimization. Patients reaching the molecular response milestones at 3, 6 and 12 months had a significantly better survival, the faster response of a treatment arm (IM800mg) did not translate into a detectable survival advantage.

Table 1.

Summary/Conclusions: Monotherapy with IM400mg provides a close to normal life expectancy. Faster response does not necessarily translate into better survival. Outcome of CML is currently more determined by disease biology and demographics than by treatment optimization.

BOSUTINIB VS IMATINIB FOR NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA: INITIAL RESULTS FROM THE BFORE TRIAL

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Background: Bosutinib (BOS) is a potent, dual SRC/ABL tyrosine kinase inhibitor approved for treatment of adults with Philadelphia chromosome-positive (Ph+) chronic myeloid leukemia (CML) resistant or intolerant to prior therapy.

Aims: To assess the efficacy and safety of BOS versus imatinib (IM) for first-line treatment of chronic phase (CP) CML in the BFORE trial (NCT02130557).

Methods: In this ongoing, multinational, phase 3, open-label study, 536 patients with newly diagnosed CP CML were randomized 1:1 to BOS 400mg once daily (n=268) or IM 400mg once daily (n=268 [3 not treated]). Informed consent was obtained from all patients. Per protocol, efficacy was assessed in a modified intent-to-treat (mITT) population of 487 Ph+ patients (BOS, n=246; IM, n=241) with the e13a2/e14a2 transcript-Ph+ patients and those with unknown Ph status and/or BCR-ABL transcript type were excluded from this population.

Results: After ≥12 months of follow-up, 78.0% of BOS and 73.2% of IM patients remain on treatment with median treatment durations of 14.1 months and 13.8 months, respectively. Major molecular response (MRM) rate at 12 months (primary endpoint) was significantly higher with BOS versus IM in the mITT population (74.2% vs 63.9%, P=0.02) as well as in the ITT population of all randomized patients (46.6% vs 36.2%, P<0.02). In the mITT population, time to MMR was shorter for BOS (hazard ratio=1.34 based on cumulative incidence; P=0.02). Rate of complete cytogenetic response (CCyR) by 12 months was also significantly higher with BOS versus IM (77.2% vs 66.4%, P=0.038), with time to CCyR shorter for BOS (hazard ratio=1.38; P<0.001). Rate of BCR-ABL transcripts ≤10% (Intl. Scale) at 3 months was higher with BOS versus IM (75.2% vs 73.3% P=0.01); rates of deep molecular response over time were also generally higher with BOS (Table). Results for molecular endpoints were similar in the ITT population.

The safety profile was consistent with the known safety profile, higher incidences of gastrointestinal events and transaminase elevations were observed with BOS. Primary results from this study suggest BOS may be an important treatment option for patients with newly diagnosed CP CML.

Summary/Conclusions: Patients on BOS had significantly higher rates of 12-month MMR and CCyR and achieved responses faster than those on IM. Consistent with the known safety profile, higher incidences of gastrointestinal events and transaminase elevations were observed with BOS. Primary results from this study suggest BOS may be an important treatment option for patients with newly diagnosed CP CML.

Figure 1.

Table 1.
CHRONIC MYELOID LEUKEMIA PATIENTS WERE NOT DIFFERENT IN MOLEULAR RELAPSE AFTER STOPPING IMATINIB IN MR4 WHERE RELAPSE WAS DETECTED OR NOT - WHEN ADJUSTING FOR NUMBER OF CONTROL TRANSCRIPTS


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Background: With imatinib (IM), most patients with chronic myeloid leukemia (CML) achieve deep molecular responses. Six months after stopping tyrosine kinase inhibitor in deep response in the EURO-SKI trial, 61% of the patients were still in molecular response at 18 months. However, in major molecular remission (0-3 log reduction in BCR-ABL1 levels) (Mahon ASH 2016). Between patients with and without BCR-ABL1, the difference in RFS at 6 months was not significant when assessing BCR-ABL1 detectability at the MR4.5 level (at least 4.9 log units from the MR4.5 threshold) (Pfrimmer ASH 2016).

Aims: For 91 of 448 patients of the EURO-SKI learning sample, the sensitivity to claim undetectable disease at the MR4.5 level was not given. Aim was to investigate whether RFS probabilities would be different when comparing detectable and undetectable disease at the MR4 level.

Methods: Detectability of BCR-ABL1 depends on the number of control gene transcripts. To reduce bias when comparing “MR4 detectable disease” (MR4 but still detectable BCR-ABL1 transcripts; i.e. 0.01- 0.0033% IS) and “MR4 undetectable disease” (MR4 without detectable BCR-ABL1) based on 10,000-31,999 ABL1 or 24,000-76,999 GUSB copies), two samples with similar sensitivity of identifying BCR-ABL1 were to be identified using propensity score (PS) matching (Rosenbaum, Rubin 1983). Apart from type (ABL1 or GUSB) and number of control gene transcripts, matching variables were interferon alpha pre-treatment, duration of MR4, and the IM treatment time before observation of MR4. Logistic regression was used to compare RFS at 6 months. Significance level was 0.05.

Results: A total of 448 patients had eligible, complete, and sufficient molecular data prior to and within the first 6 months after stopping IM treatment. All molecular results had sensitivity at the MR4 level with yet detectable disease in 196 patients (44%). With small differences in GUSB copy numbers (used in 96 of 448 cases, i.e. 0.01- 0.0033% IS) and “MR4 detectable disease” (MR4 but still detectable BCR-ABL1 transcripts; i.e. 0.01- 0.0033% IS) and “MR4 undetectable disease” (MR4 without detectable BCR-ABL1) based on 10,000-31,999 ABL1 or 24,000-76,999 GUSB copies), two samples with similar sensitivity of identifying BCR-ABL1 were to be identified using propensity score (PS) matching (Rosenbaum, Rubin 1983). Apart from type (ABL1 or GUSB) and number of control gene transcripts, matching variables were interferon alpha pre-treatment, duration of MR4, and the IM treatment time before observation of MR4. Logistic regression was used to compare RFS at 6 months. Significance level was 0.05.

Summary/Conclusions: In conclusion, we propose that the megakaryocytic differentiation arrest and self-renewal controlled by ETO2-GLIS2 results from imbalance of master transcription factors imposed by aberrant chromatin structures at enhancers that may be disrupted by targeting the NHR2 interface.

AML Biology II: Epigenetic targets

S427

ETO2-GLIS2 RECRUTS ETO2/ERG COMPLEX AT SUPER-ENHANCERS TO CONTROL TRANSCRIPTION AND DRIVE LEUKEMIC PROPERTIES IN PEDIATRIC ACUTE MEGAKARYOBLASTIC LEUKEMIA

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Methods: We first defined the consequences of ETO2-GLIS2 expression on hematopoietic progenitors and the contribution of ETO2 and GLIS2 on differentiation and self-renewal by methylcellulose replating assays and phenotype characterization. We then assessed global expression profiling and ETO2-GLIS2 direct binding on DNA by ChIPseq experiments. With immunoprecipitation experiments, we identified some ETO2-GLIS2 complex members. Finally, we tested the effects of a small peptide that could inhibit ETO2-GLIS2 complex stabilization both in vitro and in vivo.

Results: We showed that the GLIS2 moiety drives the megakaryocytic phenotype whereas both the ETO2 and GLIS2 moieties are required for maintaining self-renewal. Global expression profiling and comparison to patients’ signature consistently identified ETO2-GLIS2-mediated deregulation of major transcriptional regulators of hematopoiesis and leukemogenesis. Especially, ETO2-GLIS2 brings on an imbalance in ETS/GATA factors illustrated by an extinction of GATA1 and an overexpression of the ERG oncogene. We identified that ETO2-GLIS2 directly binds DNA via ETO2 complexes and through its GLIS2 moiety. Moreover, the ETO2-GLIS2 fusion localizes at half of H3K27ac-dense enhancers, so called super-enhancers, to control transcription of associated genes, in close association with ERG. Dimerization of ETO2-GLIS2 and interaction with endogenous ETO2 via its NHR2 domains were demonstrated with chromatin immunoprecipitation experiments. This interaction inhibited the oligomerization, reversed the transcriptional activation at enhancers, promoted megakaryocytic differentiation and abrogated human AMKL cells maintenance in vivo. So, the interaction of ETO2-GLIS2 with ETO2 complexes is an essential node for the transcriptional control by the fusion at enhancer elements. Finally, ETO2 is localized at super-enhancers and is involved with up-regulation of associated genes. ERG knockdown or genetic inactivation downregulates expression of ETO2-GLIS2 targets required for leukemic cells survival. Together, the strong up-regulation of ERG by the fusion and the presence of ERG at super-enhancers suggest a feed forward mechanism to impose gene deregulation.

Summary/Conclusions: In conclusion, we propose that the megakaryocytic differentiation arrest and self-renewal controlled by ETO2-GLIS2 results from imbalance expression of master transcription factors imposed by aberrant chromatin structures at enhancers that may be disrupted by targeting the NHR2 interface.

S428

NUCLEOSIDE BINDING PROTEIN HMGN1 BLOCKS MYELOIDOIFFERNTIATION AND PROMOTES CLONAL DOMINANCE VIA ABBERRANT HISTONE H3K27 METHYLATION

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Background: Acute myeloid leukemia (AML) is characterized by rapid growth and block in differentiation of myeloid progenitors. The AML blast is defined by having “open” chromatin. We hypothesized that alterations of chromatin compaction may promote AML. Reversing those changes could represent a novel therapeutic approach.

Aims: Gain of chr21q22 is the most common focal amplification in complex karyotype AML. HMGN1 is a chromatin-regulatory protein on 21q22 known to affect lymphoid development, and our preliminary data suggested that HMGN1 could directly mediate a myeloid differentiation block. Since HMGN1 is known to decompact chromatin and alter histone marks, our goal was to define and therapeutically target the mechanisms by which HMGN1 overexpression disrupts myeloid differentiation and promotes clonal dominance.

Methods: We immortalized bone marrow progenitors from wild-type (WT) or OE-HMGN1 mice (transgenic overexpressing HMGN1) with an estrogen receptor-HoxB8 fusion protein. Using exogenous estrogen to control nuclear translocation of HoxB8, we analyzed synchronized myeloid differentiation by flow cytometry, RNAseq, and TMT proteomics analysis. We performed MINT-ChIP-seq (M Nasase Indexed T7-chromatin IP) to measure the histone marks H3K27ac, H3K27me3, H3K4me3 and total Histone H3. We also measured histone marks in hematopoietic stem and progenitor populations in vivo. We performed competitive bone marrow transplantation with CD45.1 WT and CD45.2 OE-HMGN1 donors and measured the relative contribution to hematopoiesis over time.

Results: Synchronized differentiation in WT cells progressed over 6 days from myeloid progenitors to mature neutrophils and monocytes, analyzed by cell surface markers, morphology, and gene and protein expression. OE-HMGN1 cells proliferated faster and remained as underdifferentiated myeloblasts (84% CD11b+Gr1+ in WT vs 4% in OE-HMGN1, p<0.002; Fig A). Gene set enrichment analysis revealed more similarity to undifferentiated hematopoiesis and leukemia signatures in OE-HMGN1 cells. MINT-ChIP indicated higher global and locus-specific levels of H3K27ac in OE-HMGN1 cells (Fig B, upper panel), consistent with an increase in gene transcription, confirmed by RNA-seq. We found a specific increase in HoxA cluster expression in OE-HMGN1 cells, highlest at HoxA7 and HoxA9, genes known to be important in AML pathogenesis. In agreement with gene expression, among the most differentially measured histone peaks genome-wide were higher H3K27ac at HoxA genes amongst all differentiation time points analyzed (Fig B, lower panel). Competitive transplantation demonstrated an advantage to OE-HMGN1 stem and progenitor cells. The clonal dominance of OE-HMGN1 over WT cells extended to all populations analyzed (long- and short-term HSCs, multipotent progenitors, CMP, GMP and MEP; Fig C) and to mature lineages (myeloid, B and T cells). MINT-ChIP in LK and LKS stem and progenitor cells proliferating in vitro (Fig B, lower panel) and expressing markers of AML blast (Fig C) revealed increased H3K27ac peaks at cell cycle and leukemia-related genes in the context of OE-HMGN1. H3K27 acetylation is catalyzed by the CBP/p300 histone acetyltransferase (HAT), suggesting that HAT inhibition could target leukemias with HMGN1 overexpression. Indeed, treatment of myeloid progenitors with the CBP/p300 inhibitor Cs46 rescued the differentiation block in OE-HMGN1 cells (93% CD11b+Gr1+ in WT vs 89% in OE-HMGN1, p<NS).

Summary/Conclusions: Our study suggests that HMGN1 overexpression blocks myeloid differentiation and promotes proliferation in hematopoietic progenitors via increased H3K27 acetylation. Targeting epigenetic changes downstream of HMGN1 or interfering with HMGN1 itself may represent a novel therapeutic strategy in AML.
Acquired and inherited platelet disorders

S431
THE COMBINATION OF ORAL ALL-TRANS RETINOIC ACID AND DANAZOL VS DANAZOL AS SECOND-LINE TREATMENT IN ADULT IMMUNE THROMBOCYTOPENIA: A MULTICENTRE, RANDOMIZED, OPEN-LABEL TRIAL

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Background: Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by increased platelet destruction and impaired platelet production. Despite decades of basic and clinical research, the treatment of severe, corticosteroid-resistant or relapsed disease remains a great challenge. Our preliminary study indicated the effectiveness of all-trans retinoic acid (ATRA) for ITP (Wang M, et al. ASH 2012, Abstract #3338). This has been coupled with previous discoveries of an immune-modulation effect of ATRA in ITP, including its role to induce changes in Treg cells (Ruan CG 2016), and to correct the imbalance of aberrant macrophage polarization (unpublished data), indicating ATRA as a potential therapeutic regimen. Danazol has been used in the treatment of ITP for more than 30 years. Apart from its haemopoietic stimulatory and immune-modulatory effect, it has recently been shown to reverse abnormal myeloperoxidase activity in patients with thrombocytopenia (Townesley DM 2016). The combination of ATRA and danazol may work synergistically based on the mechanism of action targeting both increased platelet destruction and insufficient platelet production.

Aims: To investigate the efficacy and safety of ATRA plus danazol in patients with corticosteroid-resistant or relapsed ITP.

Methods: A multicentre prospective study was performed in non-splenectomized corticosteroid resistant/relapsed ITP patients. Participants were at least 18 years of age, had a platelet count of less than 30×109/L at enrolment, and did not achieve a sustained response to treatment with full-dose corticosteroids for a minimum duration of 4 weeks or relapsed during steroid-tapering or after its discontinuation. Written informed consents were obtained from all of the participants. The primary endpoint was a sustained response. The secondary endpoints included overall response, time of response, duration of response, incidence of bleeding symptoms and safety.

Results: From 2012 to 2016, 130 consecutive patients were enrolled from 5 different tertiary medical centres in China. Thirty-seven patients were ineligible and excluded, leaving 93 patients randomized to the ATRA+danazol group (n=45) and the danazol group (n=48). At 12 months' follow-up, sustained partial or complete response was achieved in 71.6% of patients in the danazol+ATRA group, significantly higher than 47.2% for danazol monotherapy (p<0.001). Additionally, 92.5% and 42.5% of patients receiving ATRA+danazol achieved at least one response (R), while only 58.3% and 11.1% of patients with danazol monotherapy achieved R and CR, respectively. In patients achieving CR or R, the median time to treatment response was 30.5 days with a peak platelet count of 155×109/L in the danazol+ATRA group compared with 49 days with a peak PLT of 69×109/L in the danazogroup. Multivariate analysis revealed that the initial response at day 28 and the median ITP duration were the potential variables associated with a sustained response. There was no treatment-related death due to adverse events. One patient receiving danazol monotherapy died from intracranial haemorrhage 4 weeks after study enrollment.

Summary/Conclusions: Our findings demonstrate that the combination of ATRA and danazol is safe and effective in achieving a rapid and long-lasting response, making it a promising promising therapeutic option for patients with novel- and/or corticosteroid-resistant or relapsed ITP. This study is registered at www.clinicaltrials.gov as # NCT01667263.

S432
NOVEL PERSPECTIVES IN GENOTYPE-PHENOTYPE CORRELATIONS IN MYH9-RELATED DISEASE: NO LONGER JUST A MATTER OF HEAD OR TAIL

TMYH9-RELATED DISEASE: NO LONGER JUST A MATTER OF HEAD OR TAIL

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Background: MYH9-related disease (MYH9-RD) is an autosomal-dominant disorder caused by mutations in MYH9, the gene for non-muscle myosin heavy
chain IIA (NMMHC-IIA), and represents the most frequent inherited thrombocytopenia worldwide. NMMHC-IIA comprises two distinct domains, the N-terminal globular head domain (HD) and the C-terminal tail domain (TD), and causative mutations hit either the HD or the TD. All patients present at birth with macrothrombocytopenia and only some of them develop during life additional manifestations, including nephropathy often leading to end-stage renal disease (ESRD), sensorineural deafness, and/or cataract. Thus, the search for genotype-phenotype correlations in MYH9-RD has been an important research topic since the identification of the disorder. In 2008, the analysis of 108 patients allowed to conclude that the mutations affecting the HD were associated with evolution to early-onset ESRD and deafness, whereas the risk of severe ESRD was much lower for patients carrying mutations of the TD. In 2014, raising to 255 the number of patients, we suggested that evolution to juvenile ESRD associated only with the most frequent among HD mutations, i.e. substitution of the arginine 702 (R702). Conversely, the presence of TD mutations were almost always associated with a distinct hydrophilic region at the interface between the SH3 subdomain and the motor domain (SH3/TD interface), may be associated with a much less severe evolution.

Aims: To improve prognostic assessment of patients with MYH9-RD.

Methods: All the consecutive patients enrolled in the Italian registry for MYH9-RD until June 2016 were included. The association of mutations identified in MYH9 gene with phenotype was assessed by a generalized linear regression model (event-free survival analysis).

Results: We enrolled 350 patients belonging to 199 MYH9-RD pedigrees. Mutational screening allowed us to identify 6 novel causative mutations in the HD of 6 different pedigrees. Interestingly, all of these variants were localized in the hydrophilic region of the SH3/TD interface. By raising the number of patients with mutations in this region from 14 to 26, and increasing the observation time, we could demonstrate that the mutations in the SH3/TD interface are associated with a milder phenotype characterized by development of hearing impairment only (“auditory” phenotype). Our study confirmed a genotype-phenotype correlation in MYH9-RD that overcomes the previously reported dualism between HD and TD mutation.

Summary/Conclusions: Mutations in the HD of the NMMHC-IIA are almost all localized in a specific region at the SH3/TD interface, which therefore represents a critical region for MYH9-RD pathogenesis. Most importantly, patients with mutations in this domain developed into two different prognostic groups: subjects with R702 substitutions are expected to evolve to a severe and early-developed disorder, whereas mutations in the SH3/TD interface are associated with evolution to a milder phenotype, characterized by development of hearing impairment only (“auditory” phenotype). Our study confirmed a genotype-phenotype correlation in MYH9-RD that overcomes the previously reported dualism between HD and TD mutation.

S434

POSITION OF THE GF1B ZINC FINGER MUTATION DECOUPLES CD34 EXPRESSION FROM ALPHA-GRANULE DEFICIENCY IN GF1B-RELATED PLATELET DISORDERS

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Background: GF1B is a transcription factor that plays an important role in haematopoiesis. Families with a mutation of the fifth DNA-binding zinc-finger domain of GF1B experience bleeding and have a platelet phenotype characterised by macrothrombocytopenia, increased CD34 expression and alpha-granule deficiency.

Aims: To explore the function of other zinc finger domains of GF1B we have characterised two unrelated families with a GF1B variant, C166F, predicted to disrupt the first C-terminal zinc finger domain and compared the phenotype with a previously described pedigree with the H294fs mutation that disrupts the fifth C-terminal zinc finger domain.

Methods: Clinical platelet phenotypes were determined by light and transmission electron microscopy and functional studies performed by light transmission and whole blood impedance aggregometry. Platelet protein expression was measured by flow cytometry and western blotting. DNA-binding of variants was determined by gel mobility shift assays (EMSA) and changes in gene transcription by luciferase assays. Cellular phenotypes were then studied in patient-specific iPSCs derived from megakaryocytes.

Results: Individuals with both C166F and H294fs are thrombocytopenic (mean platelet count =107 x109/L, n=8) but lack the collagen induced aggregation defects and bleeding symptoms observed in individuals with H294fs (ISTH BAT, P=0.015). Alpha granule content observed by microscopy and quantitated by western blotting of granule related proteins, α-spectrin and fibrinogen, were similar between C166F and control platelets and this was significantly greater than that observed for the H294fs mutation (P<0.01). EMSA studies indicate that the C166F variant retains the ability to bind DNA whereas the H294fs mutation altering Zn finger 5 abrogates DNA binding. Despite retaining the ability to bind DNA, the C166F variant de-represses genes transcription at TUBB1, TUBB2 and GF1B target genes (P<0.01). This de-repression was less marked than that observed with the non-DNA-binding H294fs mutation (P<0.01). The transcriptional de-repression observed at the CD34 promoter with both Zn finger domain 1 and 5 variants was validated by an increase in platelet surface CD34 measured by flow cytometry and total CD34 protein measured by western-dimension of GF1B and TUBB1 in human megakaryocytes.

Summary/Conclusions: Mutations altering GF1B Zn finger 1 cause thrombocytopenia with increased CD34 expression but these platelets retain repressive transcription of alpha-granule targets. Platelet protein expression was less marked than that observed in platelets derived from individuals with FL11, RUNX1 or MYH9 mutation. To validate these clinical observations, iPSC cultures were generated from the different pedigrees and megakaryocyte differentiation performed in vitro. Megakaryocyte CD34 expression was increased in cells derived from individuals with both C166F and H294fs variants but alpha granule deficiency was only observed in cells containing the non-DNA-binding H294fs mutation.
Acute lymphoblastic leukemia - Biology

S435

The significance of JAK signaling in DS-ALL

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Background: JAK signaling is critical for hematopoietic cell proliferation and differentiation.

Methods: Genomic analysis of 25 matched diagnosis remission and relapse DS-ALLs was performed using whole exome sequencing.

Results: Genomic analysis of 25 matched diagnosis remission and relapse DS-ALLs revealed lesions affecting known driver genes in the samples. In 80% of the 25 DS-ALLs, pharmacological inhibition and genetic CRISPR-mediated silencing of JAK2 diminished viability of tumor cell lines.

Summary/Conclusions: JAK2 is a critical driver of DS-ALL tumorigenesis and is a potential therapeutic target.

References:


2. The TREATMENT OF PRIMARY ADULT CHRONIC IMMUNE THROMBOCYTOPENIA (CITP) WITH FOSTAMATINIB, AN ORAL SYK INHIBITOR: RESULTS OF TWO RANDOMIZED, PLACEBO-CONTROLLED PHASE 3 STUDIES


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Background: ITP is characterized by autoantibody-directed platelet destruction mediated by activated monocyte Fc receptors which signal via spleen tyrosine kinase (syk). A Phase 2 trial of the oral syk inhibitor Fostamatinib (FOSTA) in 16 patients (pts) with refractory ITP provided preliminary efficacy and safety data (Podolanczuk et al., 2009).

Aims: To evaluate the efficacy and safety of FOSTA in adult cITP in 2 parallel, identical, multi-center, randomized, double-blind phase 3 studies (S047 and S048) of 24 weeks duration, followed by an open label study (S049).

Methods: 150 pts with 3 platelet (plt) counts (<30K/μL) were enrolled (76 in S047, 74 in S048) with a 2:1 randomization to FOSTA 100mg or placebo bid, stratified by gender, baseline plt ct <15K/µL, prior TPO-RA or splenectomy did not substantially affect response. In S049, 9/41 (22%) pts newly treated with FOSTA have achieved an IR, making the overall response rate 29% (29/101) for FOSTA pts (p=0.007); 11 additional FOSTA and no placebo patients were in potential remission.

Results: Across both studies, a SR occurred in 18/101 (18%) FOSTA vs 1/49 (2%) placebo pts, and no placebo patients achieved an IR, making the overall response rate 29% (29/101) for FOSTA vs 2% (1/49) for placebo (p<0.0001). The median plt counts were 95K, 49K, 20.5K and 17.5K/μL in SR, IR, non-responders (NR) and placebo pts, respectively. In SR and IR, median time to first plt ct ≥50K/μL was 2 weeks. Age (< or ≥65 y), gender, baseline plt ct (<15K/μL), prior TPO-RA or splenectomy did not substantially affect response. In S049, 9/41 (22%) pts newly treated with FOSTA have a SR, consistent with S047 and S048. Forty-five of 101 (54%) FOSTA pts and 14/49 (29%) placebo pts had a plt increase ≥20K/μL (p=0.005). Three of 18 (17%) SR and 1/11 (9%) IR to FOSTA compared to 26/72 (36%) NR and 22/49 (45%) of the placebo group received ≥1 rescue medication, respectively. In S047-S048, serious bleeding occurred in 5.6% of the SR and 10.2% of placebo pts, but not in the 29 responders. The number of pts with ≥1 adverse event (AE) was similar in FOSTA vs placebo (83% vs 75%). The majority AEs in FOSTA were mild or moderate; all resolved over time. Most common AEs were: diarrhea (29% vs 19%), nausea (19% vs 8%), hypertension (20% vs 8%), ALT/AST increase (10% vs 2%). Serious AEs were reported in 13% FOSTA vs 21% placebo pts.

Summary/Conclusions: Fostamatinib substantially improves plt cts in certain pts with heavily pre-treated, severe cITP of long disease duration. AEs are mostly mild or moderate in severity. Given its unique mechanism of action based on inhibition of syk, FOSTA could, if approved, be an important alternative as single agent and be a useful component of combination therapy for pts with difficult cITP.

References:

Tumor Necrosis Factor Receptor 2 Is Required for RIP1-Dependent Cell Death in Leukemia

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Background: Persistence of residual leukemia cells, due to deficiencies in apoptotic programs, is a major driver of relapse. Activation of alternative non-apoptotic cell death pathways such as necroptosis represents an attractive strategy to eliminate residual leukemia cells and prevent relapse. We have previously shown that SMAC-mimetics (SM) potently induce cell death by simultaneous RIP1-dependent apoptosis and necroptosis in a subset of refractory acute lymphoblastic leukemia (B-ALL) patient-derived samples. The molecular signals that drive sensitivity to RIP1-dependent cell death remained elusive so far.

Aims: The aim of this project was to understand the mechanisms that determine the specific vulnerability to necroptosis in ALL.

Methods: To identify molecular determinants of sensitivity to SM, we correlated the gene expression profiles of 17 primary samples with high and low sensitivity to SM with the IC50 in response to two SM compounds, birinapant and LCL161. We confirmed the top scoring genes including TNF receptor 1 (TNFR1) and TNFR2 by quantitative RT-PCR in patient-derived xenografts. We further validated our results by quantifying the expression of the candidate genes in an independent cohort of relapsed primary B-ALL and by screening samples with different expression levels of TNFR1 and 2 for their response to SM in vitro. To assess the mechanistic role of TNFR1 and 2 in the response to SM, we generated patient-derived TNFR1 and TNFR2 knockout cells using the CRISPR/Cas9 gene editing technology, and evaluated their response to SM in vitro and in vivo using a CRISPR selection model. Additionally, we overexpressed TNFR2 and evaluated the cell death phenotype. To determine the mechanism of TNFR2-mediated sensitization to SM, we investigated the formation of the pro-death RIP1-TNFR1 complex in wild type versus TNFR2ko and in SM sensitive and resistant ALL by immunoprecipitation in primary ALL samples.

Results: Comparative gene expression profiling indicated a correlation of the expression of TNFR2 with sensitivity to SM in primary ALL. Using an independent cohort of relapsed ALL samples, we found that high TNFR2 expression predicted sensitivity to SM in an ex vivo model of the bone marrow. Deletion of either TNFR1 or TNFR2 using CRISPR/Cas9 in patient-derived ALL conferred resistance to treatment with SM in vivo in the xenograft model, indicating that TNFR1 and 2 are both functionally required for cell death. In agreement with an important role for TNFR2 in the response to SM, the overexpression of TNFR2 leads to increased sensitivity to the TNFR1/RIP1 death axis. On the mechanistic level, recruitment of RIP1 to TNFR1 is a key event in the activation of cell death, which is abolished in TNFR2-deficient leukemia and does not occur in SM resistant cases.

Summary/Conclusions: Taken together, our data reveal a novel function of TNFR2 in cell death signaling, as TNFR2 predicts sensitivity to SMAC mimetics and plays a key role in activating the TNFR1/RIP1 cell death pathway, which underlies the switch from RIP1-controlled cell death to cell death and characterizes a distinct vulnerability in ALL.

THERAPEUTIC TARGETING OF ONCOGENIC MYB ACTIVITY IN T-ALL

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Background: T-lineage acute lymphoblastic leukemia (T-ALL) is an aggressive hematologic malignancy that accounts for 10%–15% of pediatric and 25% of adult ALL cases. The prognosis of T-ALL has gradually improved, however, the outcome of T-ALL patients with primary resistant or relapsed leukemia remains poor. Thus, further advances in the treatment of T-ALL will require the development of effective and highly specific molecularly targeted antileukemic drugs. The proto-oncogene MYB (encodes c-MYB) is aberrantly activated in a subset of T-ALL patients through T-cell receptor driven translocations or genomic duplications of the MYB locus itself. Recently, a new genetic mechanism for the generation of oncogenic super-enhancers in malignant T cells was identified, and suggests a general role for MYB in the regulation of T-cell specific super-enhancer activity.

Aims: We want to identify the role of enhanced MYB activity in super-enhancer driven oncogenic transcription in the context of malignant T-cell development and investigate the in vivo role of cMyb in the initiation and maintenance of T-ALL.

Methods: To evaluate if cMyb could act as a bona fide oncogene in the pathogenesis of T-ALL, we developed a conditional R26-driven cMyb overexpression mouse model. For this, we used a targeting vector that contained a floxed stop cassette followed by the cMyb gene and a EGFP/luciferase reporter, which was subsequently targeted in mESCs using recombinase-mediated cassette exchange (RMCE).

Results: Here, we report a novel conditional Myb knockin mouse model (R26-Myb). To study the in vivo oncogenic capacity of Myb, we initially crossed this conditional Myb knockin model with VaviCre mice, in order to obtain hematopoietic specific expression of Myb and the EGFP/luciferase from the ROSA26-promoter. Notably, Vav-CreERT2 R26-MybR26 mice developed T-cell lymphomas with a median latency of 77 weeks, suggesting that Myb can act as a bona fide oncogene in malignant T-cell transformation (Figure 1A). Next, we crossed our Myb transgenic model with Pten conditional knockout mice, to allow comparative analysis of tumors with and without T-cell specific Myb expression. Genetic inactivation of Pten is frequently observed in human T-ALL, and T-cell specific deletion of Pten (using Lck-Cre) results in T-cell leukemia/lymphoma development with an average of 17 weeks. Using this strategy, we obtained mice that overexpress R26-driven cMyb and lack Pten in developing T-cells and found that cMyb expression synergizes with Pten deletion, resulting in fully penetrant and accelerated T-ALL formation (median survival of 84 days instead of 118; p = 0.0003; Figure 1B). Finally, we used this novel murine T-ALL model to identify new therapeutic strategies for MYB dependent T-ALL. Importantly, the tumor cells from the cMyb knockin mice are luciferase-positive and are therefore suitable for in vivo drug testing using bioluminescence. Using this model, we evaluated the in vivo anti-leukemic efficacy of a variety of small molecules and identified new drugs that impede Myb protein stability or Myb-mediated transactivation in Myb driven tumorigenesis.

Summary/Conclusions: We developed a novel Myb-driven T-ALL mouse model and could demonstrate a pathogenic role for cMYB in T-cell leukemia. In addition, the Myb-driven preclinical mouse model will open new avenues for therapeutic intervention in T-ALL.
of -1 PRF can however only partially explain observed JAK-STAT protein Stat genes and observed RPL10 R98S associated frameshifting reduction in of cytokine receptors. We identified -1 PRF signals in mouse and human Jak-R98S and JAK-STAT lesions, suggesting that RPL10-R98S also modulates the as well as increased sensitivity of these cells to clinically used JAK-STAT pathway activation upon cytokine stimulation in RPL10 R98S knock-in mouse model and in material derived from xenografted T-ALL patient samples.

Background: Several somatic ribosome defects have recently been discovered in cancer, yet their underlying oncogenic mechanisms remain poorly understood. Alterations in ribosomal protein genes RPL5, RPL10, and RPL22 have been described in ~20% of T-cell acute lymphoblastic leukemia (T-ALL) cases. Whereas RPL5 and RPL22 show heterozygous inactivating mutations and deletions, RPL10 contains a clear mutational hotspot at residue arginine 98 (R98), with 8% of pediatric T-ALL patients harboring this RPL10 R98S missense mutation. Aims: Investigating the pathogenic role of the recurrent R98S mutation in ribosomal protein L10 (RPL10) in T-ALL. Methods: A label-free quantitative proteomics experiment was performed to screen for differentially expressed proteins in engineered mouse lymphoid Baf3 cells expressing RPL10 WT or RPL10 R98S. Differences in protein expression were further validated in hematopoietic cells derived from a transgenic RPL10 R98S knock-in mouse model and in material derived from xenografted T-ALL patient samples.

Results: The differential proteome screen revealed overexpression of several Jak-Stat signaling components (Csf2rb/2, Jak1, Stat1, Stat3, Stat5a/b and Stat6) in engineered RPL10 R98S mouse lymphoid cells, which we confirmed in hematopoietic cells derived from a transgenic RPL10 R98S knock-in mouse model and in material derived from xenografted T-ALL patient samples.
Thrombotic disorders

ASSESSING THE RISK-BENEFIT OF ANTICOAGULANTS IN ELDERLY PATIENTS WITH CANCER-ASSOCIATED VENOUS THROMBOEMBOLISM: A POPULATION BASED STUDY

Background: Cancer patients have a higher risk of venous thromboembolism (VTE) which conveys a higher subsequent mortality risk; conversely, they also have a higher risk for bleeding due to many factors including abnormal tumor anatomy and the use of chemotherapy agents with the associated risk for thrombocytopenia. However, the consequences of a recurrent VTE or a major bleeding event (MB) might be different in terms of mortality. As a result, the risk of VTE recurrence or a MB event might bear different weights. A previous systematic review has suggested that the case fatality rates of VTE recurrence and MB are similar. However, heterogeneity in study design, outcomes and in particular the types of populations included, limited the interpretation and applicability of the results. Clinical decision making uses estimations of risk and benefit for any given intervention. In the case of VTE, anticoagulants are the cornerstone of treatment having a proven benefit in reducing the risk of recurrent VTE events with an associated increase in the risk of bleeding. Therefore, determining the risk-benefit of anticoagulants might allow for better informed treatment decisions, in particular in a population at high risk for both ends of the spectrum. Therefore, herein we sought to estimate the risk and benefit of anticoagulant therapy in cancer patients developing a VTE using data from administrative databases.

Methods: The study population consisted of individuals diagnosed with NHL in Sweden 1980-2013 (n=40,354), and up to four matched controls (n=115,677). The risk of the first thrombosis was evaluated after the diagnosis of NHL (and corresponding date for controls) and the ones that occurred less than 30 days prior to diagnosis of NHL. Kaplan-Meier survival analysis was used to estimate the risk of thrombosis and a log-rank test performed to assess statistical significance. Cox regression analysis was used to calculate hazard ratios (HRs) and 95% confidence intervals (CI) (adjusting for age, sex, year of diagnosis, and previous history of thrombosis). Risk of deep vein thrombosis, pulmonary embolism and arterial thrombosis was evaluated. Arterial thrombosis was defined as cerebral, transient ischemic attack, angina pectoris, myocardial infarction, and arterial embolism and thrombosis.

Results: NHL patients had a statistically significant increase in risk of any type of thrombosis compared to controls (HR: 1.58; 95% CI: 1.53-1.62). The risk was significantly increased for all three types of thrombosis: deep vein thrombosis (HR: 2.33; 95% CI: 2.31-2.35), pulmonary embolism (HR: 1.86; 95% CI: 1.82-1.91) and arterial thrombosis (HR:1.20; 95% CI: 1.16-1.23). The risk of thrombosis did not change during the study period for the NHL patients. There was an increased risk of thrombosis for NHL patients when compared to controls and to study time trends in the risk of thromboembolism (HR: 1.64; 95% CI: 1.59-1.69) no previous history, HR: 1.43; 95% CI: 1.37-1.50 if previous history of thrombosis). The incidence of thromboembolism for NHL patients started to increase about five months before the diagnosis of NHL, and reached its peak a month before diagnosis. The incidence stayed increased for the first year after diagnosis.

Summary/Conclusion: In this large, population-based, study based on more than 40,000 patients with NHL and almost 116,000 controls, we demonstrated that there is an increased risk of thrombosis in patients with NHL when compared to controls. This is true for all types of thrombosis. We therefore conclude that hypercoagulability seems to increase with diagnosis of NHL. Several factors may contribute to this prothrombotic state, including chemotherapy and other treatment related factors as well as the disease itself. Considering that the increase in the incidence of thrombosis was highest before and around the time of diagnosis for NHL patients, that indicates that the tumor itself may have a great impact on the hypercoagulability of these patients.
and validation cohorts. The ThroLy model was developed using data solely from a derivation cohort, which included 1236 patients. Variables were evaluated by univariate logistic regression analysis, while the model was developed using a stepwise multivariate logistic regression analysis. Once a final model was defined, patients were divided into low risk and at risk groups. The final model was assessed in the validation cohort (584 patients). The studied population was also divided, based on Khorana and Padua score, into low risk and at risk groups.

**Results:**
The study population included 1820 eligible lymphoma patients. The mean patient’s age was 53.1 years (range, 15–87 years). Most patients (83%) were newly diagnosed and had advanced stage disease: Ann Arbor stage III, 14.7% and stage IV, 44%. A total of 778 patients (42.7%) had high-grade lymphoma; 351 (19.3%) had low-grade lymphoma; 266 (14.6%) had HL; 156 (8.6%) had other forms; and 269 (14.8%) had CLL/SLL. Of all the patients included in the study, 99 (5.4%) developed at least one TE during the follow-up period. There were 73 patients with venous TE (73.7%), and 25 with arterial TE (25.3%), while 1 patient had both. Patients with aggressive NHL had significantly higher odds of developing TE compared to patients with any other lymphoma type (RR=1.5; 95% CI for RR 1.1–2.4; p=0.027). The incidence of thromboembolism was 81 (5.3%) in the newly diagnosed patients and 18 (6.2%) in relapsed patients. Overall, 35.4% (35/99) of the patients with thromboembolism experienced the event before the start of chemotherapy. The majority of patients (64.6%) had TE events during chemotherapy or within 3 months after chemotherapy. For patients classified at risk according to ThroLy score in derivation cohort, the model produced negative predictive value (NPV) of 98.5%, positive predictive value (PPV) of 25.1%, sensitivity of 75.4%, and specificity of 87.5%. In validation cohort PPV for Throly score was 28.9%. Padua and Khorana score had PPV of 15.5% and 14.8% in derivation, and 11.5% and 14.8% in validation cohort, respectively.

**Summary/Conclusions:** Lymphoma patients are at increased risk of thromboembolic events but thromboprophylaxis in these patients is largely underused. ThroLy score is more specific for lymphoma patients than suggested Padua and Khorana score, but external validation in large prospective cohort studies is required.

**Table 1. Clinical features in Group1, Group 2 and both.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Median Age (Range)</th>
<th>Mean Age (Range)</th>
<th>Standard Deviation</th>
<th>At Risk</th>
<th>VTE Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>50.4 (18–87)</td>
<td>51.9 (18–87)</td>
<td>11.6</td>
<td>14.3%</td>
<td>3.9%</td>
</tr>
<tr>
<td>Group 2</td>
<td>50.4 (18–87)</td>
<td>51.9 (18–87)</td>
<td>11.6</td>
<td>14.3%</td>
<td>3.9%</td>
</tr>
<tr>
<td>Both Groups</td>
<td>50.4 (18–87)</td>
<td>51.9 (18–87)</td>
<td>11.6</td>
<td>14.3%</td>
<td>3.9%</td>
</tr>
</tbody>
</table>

**Summary/Conclusions:** The new e-alert system further increases the use of VTE prophylaxis in hospitalised cancer patients, although this was not associated with a reduction in the VTE incidence. A relevant number of VTE events occur despite prophylaxis with standard LMWH. Identification of risk factors for thromboprophylaxis failure is needed.

This work has been funded by a biomedical research grant with the Laboratory of Pharmaceutical ROVI and an aid to the research projects of the Instituto de Salud Carlos III and the FEDER (PI13 / 01029).

**S445**

**IDENTIFICATION OF A NEW AND RELATIVELY FREQUENT SERPINC1 GENE DEFECT CAUSING ANTITHROMBIN DEFICIENCY HARDLY DETECTED BY CURRENT MOLECULAR METHODS: DUPLICATION OF EXON 6**

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**Background:** Antithrombin (AT) deficiency was the first thrombophilia described 50 years ago and so far the strongest one. Up to 78% of cases are explained by point mutations or small deletion/insertions in exons or flanking regions of SERPINC1 that are easily detected by sequencing analysis. A low proportion of cases (2%) is explained by gross gene defects, mainly deletions, which are detected by multiplex ligation-dependent probe amplification (MLPA) analysis. However, the molecular base of AT deficiency is unknown using current methods in 20% of cases.

**Aims:** To identify new SERPINC1 defects causing AT deficiency.

**Methods:** We studied 271 unrelated cases with AT deficiency. Functional and biochemical assays characterized plasma AT. Genetic analyses involved Sanger and Next Generation Sequencing (NGS) (PGM, Ion Torrent), MLPA and specific PCR designs.

**Results:** Sanger sequencing of PCR amplicons with primers flanking the 7 exons and further analysis with SeqscapeTM detected pathogenic mutations in 173 cases. Whole gene sequencing identified 5 mutations in regulatory regions. MLPA analysis revealed 5 cases with whole or partial deletion of the gene. Moreover, 13 cases had disorders of glycosylation. Interestingly, the analysis of the PCR product and the electropherogram of exon 6 of a 42-year-old male patient (P1) with deep venous thrombosis and 75% of anti-FXa activity

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**Background:** One-hematologic hospitalised patients constitute a group at high risk of venous and/or arterial thromboembolism (VTE). Current clinical practice guidelines recommend prophylaxis with low molecular weight heparin (LMWH) during hospitalisation, unless contraindicated. However, its underuse is a worldwide problem. Electronic alert systems (e-alerts) can improve the use of appropriate thromboprophylaxis and reduce the incidence of VTE.

**Aims:** To evaluate the impact of a new version (v2.0) of our e-alert system for VTE prevention compared with the initial software version. Secondary endpoints try to identify predictive factors for prophylaxis use and thrombotic events.

**Methods:** Prospective study including consecutive adult cancer patients admitted at our centre. From April 2014 to June 2015 (first period) the initial e-alert system version remained operative and from July 2015 to December 2016 (second period) the new version was active. The v2.0 displayed a second window that asked physicians about the reason why LMWH was not prescribed. The main outcomes were: VTE (confirmed by objective methods), clinically relevant bleeding, and mortality. All patients were followed-up during hospitalisation and 30 days after discharge. Descriptive statistical analysis and correlation between clinical variables and main outcomes were performed by using the software package SSPS V20.

**Results:** 1251 patients were included, 782 patients in the first period and 469 in the second one (main clinical features are shown in Table 1). E-alerts v2.0 was associated with an increase of appropriate LMWH prophylaxis during hospitalisation (65.2% vs 72.2%, p=0.015). However, this improvement did not result in a reduction of VTE during admission or follow-up (2.3% vs 2.3%; p=0.89). Interestingly, almost 80% of VTE events occurred despite LMWH use. No differences in the rate of major bleeding (2.8% vs 3.2%; p=0.83), and mortality (10.6% vs 14.3%; p=0.07) were observed, either. The main reason for not prescribing LMWH prophylaxis was bleeding risk, but in 17% of cases physicians did not consider that the patient really had a high VTE risk. No significant correlation was found between any of the clinical variables analyzed and the risk of VTE. Prophylaxis use was more frequent among patients with solid cancer (vs hematologic), advanced stage, active chemotherapy treatment and longer hospital stay.

**Summary/Conclusions:** The new e-alert system further increases the use of VTE prophylaxis in hospitalised cancer patients, although this was not associated with a reduction in the VTE incidence. A relevant number of VTE events occur despite prophylaxis with standard LMWH. Identification of risk factors for thromboprophylaxis failure is needed.

This work has been funded by a biomedical research grant with the Laboratory of Pharmaceutical ROVI and an aid to the research projects of the Instituto de Salud Carlos III and the FEDER (PI13 / 01029).
with no apparent gene defect by either Sanger sequencing of 7 exons or by NGS analysis of the whole gene using the Ion Torrent platform, revealed a 193 bp insertion, which corresponded to a tandem duplication involving exon 6. Family studies revealed the same duplication in 5 relatives, all with AT deficiency (60-75%). The first MLPA analysis of this case failed to detect the duplication and only after a fine readjustment, it was detected. MLPA analysis under the new conditions of the remaining 59 cases with unknown molecular base for their AT deficiency identified one additional case, P2, with potential duplication of exon 6. P2 was a 17 year-old female with 41% of anti-FXa activity, who developed deep venous thrombosis. Sanger and NGS sequencing also failed to detect any genetic defect in P2. A set of primers specific to detect tandem duplications of exon 6 was designed with forward primer from 3’ end of exon 6, and reverse primer from 5’ of exon 6. This set of primers only rendered amplification in the two cases with exon 6 duplication. The presence of 6 Alu elements up and down the gene deletions involved breakpoints affecting intron 5 (deletion of exons 2-5).

Summary/Conclusions: Our study identified a new and relatively frequent SERPINC1 gene defect causing AT deficiency that is hardly identified by current molecular methods: duplication of exon 6. This genetic defect was detected in 1% of our cohort, and represents nearly half of the total gross gene defects causing AT deficiency. The small size of this exon makes difficult the identification of this defect by MLPA. The presence of 6 Alu elements up and downstream exon 6 makes this region a hotspot for unequal recombination that may cause deletions, tandem duplications and potentially transpositions, which may produce AT deficiency (both severe and mild) by an aberrant splicing. We also developed a simple and specific method to detect duplications in tandem of exon 6.

Stem cell transplantation - Experimental

S446

CYTOSOLIC NUCLEIC ACID SENSORS PROMOTE INTESTINAL EPITHELIAL INTEGRITY DURING ACUTE TISSUE DAMAGE AND PROTECT FROM GRAFT-VERSUS-HOST DISEASE

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Background: The epithelial lining of the gastrointestinal (GI) tract represents the first line of defense mediating protection from microbial challenge. Next to producing antimicrobial molecules, Paneth cells contribute to this defense by providing a supportive niche for intestinal stem cells (ISCs) maintaining the epithelium. Loss of intestinal barrier function by total body irradiation (TBI) or chemotherapy (CTx) is an essential step in enhancing the development of immune-mediated regenerative strategies to promote epithelial barrier function to enhance healing after genotoxic tissue damage. CD4 T cells directed against mismatched HLA-DP can cause Graft-versus-Host Disease (GVHD) after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Our current limited ability to protect epithelium and promote regeneration in GVHD is at least in part due a limited understanding of ISC function and epithelial regeneration in the allo-HSCT setting. Recent work suggests a protective function of Type I Interferons (IFN-I) at epithelial surfaces and in the prevention of GVHD. Yet, the molecular pathways that trigger those functions during acute tissue damage are poorly understood. Given that the RIG-I-MAVS and STING pathways are important regulators of IFN-I production and IFN-Is can initiate epithelial repair, we hypothesized that activation of these pathways during conditioning therapy may protect epithelial integrity and could be exploit-ed interventionaly to promote intestinal barrier function and prevent GVHD.

Aims: We aimed at characterizing the role of RIG-I-MAVS and STING during allo-HSCT, and at understanding mechanisms by which activation of these pathways can promote barrier function to enhance healing after genotoxic tissue damage.

Methods: We used an integrated approach with pathophysiologic mechanistic studies on IECs in experimental mouse models (MHC-mismatched and minor histocompatibility antigen (miHA)-mismatched transplants to model highly aggressive GVHD; genotoxic stress induced by TBI and CTx) and evaluation of immune-mediated regenerative strategies to promote epithelial barrier function (organoid cultures, barrier function test)

Results: Mice lacking MAVS were more sensitive to total body irradiation (TBI)- and chemotherapy induced intestinal barrier damage, and, like RIG-I-deficient mice, developed severe worse graft transplantation (allo-HSCT). This phenotype was not associated with changes in the intestinal microbiota, but with reduced epithelial integrity and regeneration. Conversely, targeted activation of the RIG-I pathway during damage promoted these processes and ameliorated GVHD. Mechanistically, IFN-I (RIG-I-induced or recombinant) could promote growth of intestinal organoid cultures and production of RegIIIγ. Importantly, our findings were not confined to RIG-I-MAVS signaling, as interventional engagement of the STING pathway also protected from loss of barrier function and GVHD and led to IFN-I-dependent intestinal organoid growth. Consistent with this, STING-deficient animals suffered from worse GVHD.

Summary/Conclusions: Our studies may have the potential to develop novel targeted therapies (i) to promote intestinal barrier integrity, (ii) to prevent the development of GVHD, and (iii) for the regenerative response of other tissues.

S447

CD4 T CELLS RECOGNIZING MISMATCHED HLA-DP AFTER ALLOGENEIC STEM CELL TRANSPLANTATION SHOW TISSUE SPECIFIC REACTIVITIES

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Background: Expression of HLA class II molecules is under non-inflammatory conditions predominantly restricted to hematopoietic cells. However, donor CD4 T cells directed against mismatched HLA-DP can cause Graft-versus-Host Disease (GVHD) after allogeneic stem cell transplantation (alloSCT) or donor lymphocyte infusions from HLA 10/10 matched but HLA-DP mismatched donors due to upregulation of HLA class II expression under inflammatory conditions. It is often assumed that allo-HLA-DP directed CD4 T cells recognize peptides encoded by household genes presented in foreign HLA-DP and that every cell that expresses these mismatched HLA-DP allele is a target for these T cells. However, in vitro experiments illustrated that allo-HLA-DP directed CD4 T cells were not always recognizing patient derived fibroblasts induced to express HLA-DP. We hypothe-sized that HLA-DP directed CD4 T cells can have tissue specificity if the presented peptides in HLA-DP are encoded by genes with tissue specific expression.
Aims: The aim of the study is to investigate whether donor CD4 T cells recognizing mismatched HLA-DP show tissue specific reactivities.

Methods: In a randomized clinical trial we treat patients 3 months after T cell depleted alloSCT from HLA 10/10 matched, HLA-DP mismatched, donors with 0.25-0.50 x 10^6/kg donor CD4 T cells to promote immune reconstitution. In 4 patients, Graft-versus-Leukemia reactivity and/or organ specific GVHD occurred after the infusion. To characterize the immune responses in these patients, 

Results: Allo-HLA-DP directed CD4 T cells showing differential recognition of target cells were found in all 4 patients. A total of 33 HLA-DPB1*04:01 reactive CD4 T cell clones were isolated from patient 1 who suffered GVHD of skin and colon, but not liver. Within these 33 clones, 3 clones recognized only hematopoietic target cells, 9 clones recognized hematopoietic, skin and colon derived target cells and 5 clones recognized hematopoietic and colon derived cells only. None of the T cell clones recognized biliary epithelial cells. From patient 2 total of 230 HLA-DPB1*03:01 reactive CD4 T cell clones were isolated, of which 27 recognized only hematopoietic target cells and 96 clones also recognized GVHD target cells with differences in tissue specificity. 32 HLA-DPB1*04:01 reactive T cell clones were found from patient 3, of which 6 recognized only hematopoietic target cells, whereas other clones again showed various tissue specificities. From patient 4, 26 HLA-DPB1*01:01 reactive T cells could be isolated which all recognized biliary epithelial cells with or without co-recognition of other target cells. In addition, also 11 HLA-DPB1*03:01 reactive T cells were isolated, again with different tissue specificities.

Summary/Conclusions: These results illustrate that donor CD4 T cells directed against mismatched HLA-DP show differential recognition of target cells including restricted specificity for cells of hematopoietic origin. Donor CD4 T cells recognizing hematopoietic target antigens in the context of patient specific HLA-DP alleles can be used to mediate tumor specific immune responses after HLA 10/10 matched unrelated stem cell transplantation.

S448 MESENCHYMAL STROMAL CELLS STIMULATE THE PROLIFERATION AND IL-22 PRODUCTION BY TYPE 3 INNATE LYMPHOCYTES

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Background: Infusion of mesenchymal stromal cells (MSCs) is a promising and increasingly applied therapy for patients who suffer from graft-versus-host disease (GVHD), a common and life-threatening complication of allogeneic stem-cell transplantsations (ASCT). The therapeutic effect of MSCs is mainly ascribed to their suppression of (alloreactive) lymphocyte proliferation and enhancement of tissue-repair activity. However, only about half of the GVHD patients benefit from MSC therapy, and which factors determine MSC responsiveness is unclear. We recently observed that relatively high frequencies of activated type 3 innate lymphoid cells (ILC3s) before and/or after ASCT were associated with a lower risk to develop GVHD, which may be related to the production of tissue-protective IL-22 by ILC3s.

Aims: To investigate if ILC3s can contribute to the therapeutic effect of MSCs, we studied the interaction between MSCs and ILC3s in vitro.

Methods: ILC3s isolated from human tonsils were CellTrace-labeled and cocultured with bone-marrow derived MSCs for 5 days in the presence of IL-2.

Results: Co-culture with MSCs significantly enhanced the proliferation of ILC3s and their IL-22 production. Reciprocally, ILC3s promoted ICAM-1 and VCAM-1 expression on MSCs. These experiments revealed that the interaction is mainly dependent on cell-cell contact or close proximity of MSCs and ILC3s. Addition of blocking antibodies against ICAM-1, VCAM-1, or their integrin ligands, did not affect ILC3 proliferation, suggesting that ILC3 stimulation is ICAM/VCAM independent. Soluble factors also contributed to the interaction, as ILC3s proliferated slightly better in the presence of MSC culture supernatant compared to IL-2 only. Based on experiments with blocking antibodies, we found IL-7 to be the likely candidate for this effect.

Summary/Conclusions: We show that via cell-cell contact and IL-7, MSCs promote the proliferation and IL-22 production by ILC3s in vitro, suggesting ILC3s may play a role in the control of GVHD upon MSC therapy.

S449 ABERRANT T CELL RESPONSES IN THE BONE MARROW MICROENVIRONMENT OF PATIENTS WITH POOR GRAFT FUNCTION AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Poor graft function (PGF) remains a life-threatening complication following allogeneic hematopoietic stem cell transplantation (allo-HSCT), and the underlying mechanisms have not yet been elucidated. Considerable evidence from murine studies has demonstrated that effective hematopoiesis depends on the specific bone marrow (BM) microenvironment, where hematopoietic stem cells reside. In this regard, we previously reported that PGF patients who had impaired BM endothelial and vascular microenvironment (BBMT 2013; BMT 2016; Oncotarget 2016; Blood 2016). Moreover, our pilot study showed that both CD4+ and CD8+ T cells found polarized towards a type 1 immune response in the BM microenvironment of PGF patients (N=10) compared to those in matched good graft function (GGF) patients(N=20) (BBMT 2016). Nevertheless, whether abnormalities of T cell subsets in the BM immune microenvironment, including Th1, Th17, Th1, Th2, Th2c cells and regulatory T cells (Tregs), are involved in the pathogenesis of PGF remains to be explored.

Aims: To compare the T cell subsets in the BM immune microenvironment, including Th1, Th1c, Th2c, Th17, Th2c cells and Tregs, between patients with PT and GGF after allo-HSCT.

Methods: This prospective nested case-control study enrolled 20 patients with mismatched related patient (BBMT, 2016). Moreover, our pilot study showed that both CD4+ and CD8+ T cells found polarized towards a type 1 immune response in the BM microenvironment of PGF patients (N=10) compared to those in matched good graft function (GGF) patients(N=20) (BBMT 2016). Nevertheless, whether abnormalities of T cell subsets in the BM immune microenvironment, including Th1, Th17, Th1, Th1c, Th2c cells and regulatory T cells (Tregs), are involved in the pathogenesis of PGF remains to be explored.

Aims: To compare the T cell subsets in the BM immune microenvironment, including Th1, Th1c, Th2, Th2c, Th17, Th1c cells and Tregs, between patients with PT and GGF after allo-HSCT.

Methods: This prospective nested case-control study enrolled 20 patients with mismatched related patient

Results: The demographic and clinical characteristics were similar between allo-HSCT patients with PGF and those with GGF. The percentages of Th1 (37% vs. 26.4%, P=0.0005) and Th2 (52.4% vs. 19%, P=0.0001) cells were significantly higher in PGF patients than in GGF patients, whereas the percentages of Th2 (0.8% vs. 2.4%, P=0.0001) and Th17 (0.5% vs. 1.1%, P=0.0001) cells were markedly lower in the GGF group than in the PGF group. PGF patients showed significantly greater Th1 cell/Th2 cell (31.6 vs. 10.8, P=0.0001) and Th17 cell/Th2c cell ratios (108.8 vs. 18.4, P<0.0001) than those for GGF patients. Moreover, a significantly higher proportion of stimulated CD4+ T cells that produced IL-17 (Th17) was found in the BM of PGF patients than in the BM of GGF patients and HD (3.7% vs. 16% vs. 1.1%, P<0.05), whereas the percentages of Tregs in PGF patients were comparable to those in GGF patients and HD, resulting in a dramatically elevated ratio of Th17 cells/Tregs in the BM of PGF patients relative to those in GGF patients (1.01 vs. 0.57, P=0.04).

Summary/Conclusions: The present study revealed that aberrant T cell responses in the BM immune microenvironment may be involved in the pathogenesis of PGF after allo-HSCT. These findings will facilitate the optimization of immune regulation strategies and improve the outcome of PGF patients post-allotransplant.

S450 HIGHER FREQUENCY OF SWITCHED MEMORY B CELLS PREDICTS THE INCIDENCE OF CHRONIC GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Aims: To investigate if ILC3s can contribute to the therapeutic effect of MSCs, we studied the interaction between MSCs and ILC3s in vitro.

Methods: ILC3s isolated from human tonsils were CellTrace-labeled and co-cultured with bone-marrow derived MSCs for 5 days in the presence of IL-2.

Results: Co-culture with MSCs significantly enhanced the proliferation of ILC3s and their IL-22 production. Reciprocally, ILC3s promoted ICAM-1 and VCAM-1 expression on MSCs. These experiments revealed that the interaction is mainly dependent on cell-cell contact or close proximity of MSCs and ILC3s. Addition of blocking antibodies against ICAM-1, VCAM-1, or their integrin ligands, did not affect ILC3 proliferation, suggesting that ILC3 stimulation is ICAM/VCAM independent. Soluble factors also contributed to the interaction, as ILC3s proliferated slightly better in the presence of MSC culture supernatant compared to IL-2 only. Based on experiments with blocking antibodies, we found IL-7 to be the likely candidate for this effect.

Summary/Conclusions: We show that via cell-cell contact and IL-7, MSCs promote the proliferation and IL-22 production by ILC3s in vitro, suggesting ILC3s may play a role in the control of GVHD upon MSC therapy.
Development of cGVHD.

Aims: We sought to determine if B-cell subsets measured around day 100 after allo-SCT predict the subsequent occurrence of cGVHD in a prospective clinical study.

Methods: Peripheral blood (PB) samples were obtained from consented patients (pts) between day 80 and 110 (D100) after allo-SCT at The University of Texas MD Anderson Cancer Center from 2012 to 2015. Only pts who had not been diagnosed with cGVHD or progression of underlying malignancy by D100 were eligible for this study. We analyzed CD19+CD20+ B cell subsets by FACS. Subsets were defined as naïve (CD27-IgD-), unswitched (CD27+IgD+), and switched (CD27+IgD-) memory cells. Receiver Operating Characteristic (ROC) curve was used to identify threshold levels of B cell % and numbers that predict the incidence of cGVHD. cGVHD diagnosis was based on the 2014 National Institutes of Health guidelines.

Results: A total of 80 pts were enrolled in the study. The median age at SCT was 49 years (range 21-75). The majority (80%) of pts received myeloablative conditioning, and 75% received tacrolimus with methotrexate or mycophenolate mofetil for GVHD prophylaxis. Diagnosis was myeloid (61%) or lymphoid (34%) malignancy in the majority of pts. Gifts source was primarily PB or bone marrow from matched unrelated (61%) or related (24%) donors. Grade 2-4 acute GVHD had occurred in 45% of pts before D100. Thirty-six percent of pts were receiving steroids at D100. Forty-seven (59%) pts had detectable bone marrow from matched unrelated (61%) or related (24%) donors. Grade 34% malignancy in the majority of pts.

Aims: To report on preliminary efficacy and safety data from the use of AG-348 in the ongoing DRIVE PK study (NCT02476916), an open-label dose-ranging trial of AG-348 in transfusion-independent adults with PK deficiency.

Methods: After providing informed consent, patients were randomized to AG-348 50mg or 300mg orally twice daily (BID) for 6 months (Core Period). At the end of the Core Period, patients can continue on treatment for another 2 years in the Extension Period. Treatment independence is defined as ≤3 units of red blood cells transfused in the 12 months preceding the first dose of AG-348 and no transfusions in the 4 months preceding the first dose. Patients are followed weekly for Weeks 1-3, every 3 weeks for Weeks 4-12, monthly for Weeks 13-24 and then every 3 months until the end of the study. Hormone and iron status are evaluated at Baseline, Week 12 and End of Core Period, and then every 6 months in the Extension Period.

Results: As of 18 Jan 2017, goal enrolment has been met and all 52 patients are evaluable for safety and efficacy; 24 have completed the Core Period and 23 are ongoing in the Core Period. Five patients discontinued from the Core Period, owing to adverse events (AEs) (n=2) or consent withdrawal (n=3). Of the 24 subjects who completed the Core Period, 21 entered the Extension Period and 20 are still on treatment; 1 was discontinued by the investigator. Patients are currently receiving doses ranging between <25mg BID and 300mg BID. As of the previous data cutoff date of 23 Sep 2016 (where N=34), AG-348 had an acceptable safety profile. AE attributable to AG-348 was observed in 20 (60%) of the 34 patients. Observed AEs included nausea, fatigue, diaphoresis, tremor, and headache. Efficacy endpoints inclusive of disease activity were observed in the 20 patients evaluable for efficacy at 23 Sep 2016.

Summary/Conclusions: AG-348 is a novel, first-in-class PK-R activator undergoing clinical testing in patients with PK deficiency. The ongoing DRIVE PK study has now met goal enrolment of 52 patients, and data from these patients will be available at the time of presentation. Chronic daily dosing with AG-348 is well tolerated and has demonstrated clinically relevant, durable increases in Hb across a range of doses from <25mg BID to 300mg BID. These data highlight the potential of AG-348 to be the first disease-altering treatment for patients with PK deficiency.
Background: Pyruvate kinase deficiency (PKD) is the most common glycolytic enzyme defect causing hereditary non-spherocytic hemolytic anemia. PKD does not have a specific curative treatment. Therefore treatment is mainly supportive, consisting of regular red blood cell transfusions, splenectomy and chelation therapy for iron overload. This does not impact on the quality of life for affected patients. Hemolytic anemia is the main indication for red blood cell transfusion, while red blood cell transfusion is the main indication for splenectomy. Patients with hemolytic anemia and iron overload may benefit from hematopoietic stem cell transplantation (HSCT).

Aims: The aims of this study were to characterize the penetrance of red cell transfusion and splenectomy in PKD patients treated by HSCT and to compare the outcome of PKD patients after HSCT with those of healthy controls.

Methods: Data from 103 patients with PKD who were treated for HSCT in 16 European and Asian centers were collected as part of the PKD-HEMORHAGE study, a retrospective multicentric series of 103 patients from 49 families.

Results: Among 103 patients, 165 red cell transfusions were required, with a median of 3 (range 1-125) per patient. Twenty-one patients (20.4%) underwent splenectomy. The median number of red cell transfusions per patient was 2 (range 1-125) and the median number of splenectomies per patient was 0 (range 0-1). The median age at the time of HSCT was 5.5 years (range 0-18). The median follow-up was 12 months (range 3-96). The cumulative incidence of death or relapse was 10% at 2 years and 25% at 5 years.

Summary/Conclusions: HSCT can be considered as a curative treatment for PKD patients with severe hemolytic anemia and iron overload. The main complications of HSCT were GvHD and graft failure. The long-term outcome of PKD patients after HSCT is promising, and further studies are needed to evaluate the impact of HSCT on the quality of life of PKD patients.

S453 HEREDITARY XEROCYTOSIS: CLINICAL AND BIOLOGICAL PRESENTATION AT DIAGNOSIS IN A RETROSPECTIVE SERIES OF 103 PATIENTS


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Background: Dehydrated hereditary stomatocytosis, also called hereditary xeroysis (HX), is a dominant non-spherocytic hemolytic anemia characterized by an increased leak of monovalent cations through the red cell membrane leading to dehydration and a shortened red cell survival. HX is difficult to diagnose because of its rarity and the heterogeneity in its clinical presentation. Aims: Our study aims to characterize the clinical and biological features at diagnosis in a retrospective series of 103 patients with HX.

Methods: HX diagnosis was based on the typical left-shifted curve of osmotic gradient echocytometry performed at CHU Bièvre from 1993 to 2016. All patients were from European origin. They were referred to our center for: chronic non-spherocytic hemolysis (30), thalassemic events after splenectomy (8), hyperferritinemia and/or a chelation therapy were noticed for 26 patients among the 55 for whom this data was available (47%), 19 patients were treated for iron overload: phlebotomy (14) and/or Deferasirox (6) and/or Deferoxamine (6) and/or Deferrinone (1). A perinatal edema history was noted in 17 (16,5%) patients. A history of thrombosis was noted in 7 (6,8%) patients, corresponding to a total number of 17 thrombotic events including post-embolic pulmonary hypertension (2), arterial events (3), pulmonary embolism (4), portal thrombosis (4), splenic infarcts (2) and deep venous throm-
CRIZANLIZUMAB, A P-SELECTIN INHIBITOR, INCREASES THE LIKELIHOOD OF NOT EXPERIENCING A SICKLE CELL-RELATED PAIN CRISIS WHILE ON TREATMENT: RESULTS FROM THE PHASE II SUSTAIN STUDY

Aims: This post-hoc analysis evaluated patients who did not experience a SCPC for the duration of the trial.

Methods: SUSTAIN was a randomized, double-blind, placebo-controlled, Phase II study (NCT01895361). Patients aged 16–65 years with SCD (including HbSS, HbSβ0–thalassaemia, and HbSβ+–thalassaemia genotypes) and 2-10 SCPC events in the previous 12 months were included. Concomitant use of HU was allowed, but not at the start of the study. Clinical trial was ongoing at the time of data analysis. The primary endpoint was the proportion of patients in the SUSTAIN study who remained SCPC event-free through the trial. Results: A total of 185 patients were included in the study (ITT population). The primary endpoint was met by 25.6% of patients receiving crizanlizumab 5.0 mg/kg compared with 16.9% in the placebo arm. In addition, 42 patients (22.1%) treated with crizanlizumab 5.0 mg/kg were SCPC event-free compared with 16.9% in the placebo arm. The difference was significant (p = 0.008). Conclusion: Treatment with crizanlizumab 5.0 mg/kg appears to increase the likelihood of adult patients with SCD being SCPC event-free while on treatment, even in high-risk subpopulations. Crizanlizumab 5.0 mg/kg was well tolerated in the 52-week SUSTAIN study (Ataga KI et al. N Engl J Med 2017;376:429-439).

Table 1. SCPC event-free patients by prior SCPC events, genotype and HU use

<table>
<thead>
<tr>
<th>SCPC events in the year prior to study</th>
<th>Crizanlizumab 5.0 mg/kg</th>
<th>Crizanlizumab 2.5 mg/kg</th>
<th>Placebo</th>
<th>N=65</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-4</td>
<td>17/42 (40.5)</td>
<td>10/41 (24.4)</td>
<td>10/41 (24.4)</td>
<td></td>
</tr>
<tr>
<td>5-10</td>
<td>7/25 (28.0)</td>
<td>2/25 (8.0)</td>
<td>1/24 (4.2)</td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbSS</td>
<td>15/47 (31.9)</td>
<td>9/47 (19.1)</td>
<td>8/47 (17.0)</td>
<td></td>
</tr>
<tr>
<td>HbSβ0–thalassaemia</td>
<td>25/58 (43.1)</td>
<td>19/58 (32.8)</td>
<td>15/58 (26.3)</td>
<td></td>
</tr>
<tr>
<td>HbSβ+–thalassaemia</td>
<td>19/41 (46.3)</td>
<td>12/41 (29.3)</td>
<td>12/41 (29.3)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>10/40 (25.0)</td>
<td>6/40 (15.0)</td>
<td>2/12 (16.7)</td>
<td></td>
</tr>
<tr>
<td>HU use</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14/42 (33.3)</td>
<td>9/44 (20.9)</td>
<td>7/40 (17.5)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>10/40 (25.0)</td>
<td>5/40 (12.5)</td>
<td>12/40 (30.0)</td>
<td></td>
</tr>
</tbody>
</table>

Results: Among the 198 patients included in the study (ITT population), 62.1% and 37.4% had experienced 2-4 and 5-10 SCPC events in the previous year, respectively, and 62.1% were taking HU at baseline. HbSS was the most common genotype (71.2%); HbSC: 16.2%; HbSβ0–thalassaemia: 6.1%; HbSβ+–thalassaemia: 5.1%; other: 1.5%. Overall, more patients in the crizanlizumab 5.0 mg/kg group (n=24/67; 35.8%) were SCPC event-free than in the 2.5 mg/kg group (n=12/66; 18.2%) and placebo (n=11/65; 16.9%) groups. In each of the prior SCPC events, SCD genotype and HU use subgroups, a greater proportion of patients treated with crizanlizumab 5.0 mg/kg were SCPC event-free compared with those in the crizanlizumab 2.5 mg/kg or placebo arms (Table 1). In sub-populations considered to be at increased risk of experiencing a SCPC (patients with 5-10 SCPC events in the previous year and/or with the homozygous HbSS genotype), a higher proportion of patients treated with crizanlizumab 5.0 mg/kg were SCPC event-free compared with those in the placebo arm (28.0% vs 4.2% and 31.9% vs 17.0%, respectively). Additionally, 33.3% of patients who were taking HU and treated with crizanlizumab 5.0 mg/kg were SCPC event-free during the study, compared with 17.5% in the placebo arm, possibly suggesting an additive effect.

Summary/Conclusions: Treatment with crizanlizumab 5.0 mg/kg appears to increase the likelihood of adult patients with SCD being SCPC event-free while on treatment, even in high-risk subpopulations. Crizanlizumab 5.0 mg/kg was effective in those who had experienced at least two SCPCs in the previous year despite taking HU, suggesting that this dose is effective as a disease-modifying agent that meets an unmet medical need.

S455
FREE IRON IN SERA OF PATIENTS WITH SICKLE CELL DISEASE CONtributes TO THE RELEASE OF NEUTrophil EXTRACELLULAR TRAPS

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Background: Chronic hemolysis is a hallmark of sickle cell disease (SCD). Hemolysis in SCD has been associated with elevated levels of heme in the circulation of both human patients and SCD mice. It was shown that TNF-α treatment induces experimental vaso-occlusive crisis (VOC) in SCD mice, associated with the formation of neutrophil extracellular traps (NETs) as shown by staining of lung sections (Chen et al. Blood 2014). In addition, the administration of DNase I to degrade NETs led to improved survival. Furthermore, it was shown that plasma from SCD patients obtained during SCD crisis induced NET formation by TNF-α primed neutrophils. Free heme was suggested to mediate the formation of NETs in SCD, as treatment of SCD mice with the heme-binding protein hemopexin (Hpx) to scavenge free heme led to reduced NET formation (Chen et al. Blood 2014).

Aims: To verify the potential therapeutic use of Hpx administration to block NET formation and the occurrence of VOC in human SCD, we aimed to determine
mine whether ex vivo Hpx addition to human SCD sera would prevent NET formation.

Methods: Patient serum and plasma samples were obtained from 32 incidents of VOC in 24 adult SCD patients, with informed consent. Moreover, steady state samples were obtained at least 4 weeks after discharge from the hospital. Patients having had a blood transfusion in the 3 months prior to admission were excluded. NET formation by human neutrophils from healthy donors was studied using confocal fluorescence microscopy and staining for extracellular DNA with the non-permeable dye Sytox Green. The presence of extracellular DNA that stains positive for citrullinated histone H3 confirmed the formation of NETs (Figure 1A).

Results: Indeed, we found that heme (ferriprotoporphyrin IX) activated neutrophils to generate reactive oxygen species and release NETs, which was prevented by addition of plasma-derived Hpx. Moreover, exposure of neutrophils to sera from patients with SCD promoted NET formation, which was significantly enhanced during VOC. However, we observed that circulating free heme levels were elevated in SCD patient serum irrespective of disease state, and serum concentrations of Hpx were reduced in both VOC and steady state compared to healthy donor serum. Strikingly, addition of Hpx in supraphysiological concentrations of Hpx were reduced in both VOC and steady state compared to sera from patients with SCD promoted NET formation, which was significantly enhanced during VOC. However, we observed that circulating free heme levels were elevated in SCD patient serum irrespective of disease state, and serum concentrations of Hpx were reduced in both VOC and steady state compared to healthy donor serum. Strikingly, addition of Hpx in supraphysiological concentrations failed to prevent the formation of NETs in all SCD sera tested. We and others (Chen et al. Blood 2014) have found that, in contrast to heme, protoporphyrin IX does not trigger NET formation, revealing that the iron atom is required for the release of NETs. This observation led us to investigate whether free iron may directly induce NET formation. When neutrophils were exposed to Fe-NTA or serum from a thalassemia patient with iron overload, NETs were formed. Scavenging of free iron by addition of the iron-chelator deferoxamine or the specific iron-binding protein apotransferrin prevented NET release (Figure 1B). Moreover, we found that sequestration of free iron prevented NET formation induced by a subset (6 out of 11 tested), but not all, sera of patients with VOC (Figure 1C and D). In addition, sickled red blood cells (RBCs) are known to bind to neutrophils in vitro. Here, we found that neutrophils released NETs in response to sickled RBCs, even in the presence of Hpx. By contrast, blocking of complement C5 activation completely prevented the formation of NETs when neutrophils were exposed to sickled RBCs (Figure 1E).

Summary/Conclusions: In summary, we observed that sequestration of free iron with the iron-chelator deferoxamine or the iron-binding protein apotransferrin in sickled RBCs was effective in more than half of the SCD patient sera, while the binding of heme to Hpx did not prevent NET release in human SCD patient sera. Therefore, we propose that targeting free iron with these iron binding compounds may be explored therapeutically to prevent or treat VOC development in SCD. Finally, complement activation in the presence of sickled RBCs activates neutrophils to release NETs, which may also contribute to VOC and SCD pathogenesis. Therefore, anti-CS IgG may represent an alternative therapeutic strategy to prevent VOC in SCD.

New drugs for rescue in relapsed/refractory multiple myeloma

S456

PHASE 3 ELOQUENT-2 STUDY: EXTENDED 4-YEAR FOLLOW-UP OF ELOTUZUMAB PLUS LENALIDOMIDE/DEXAMETHASONE VS LENALIDOMIDE/DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA


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Background: Elotuzumab is an immunostimulatory monoclonal antibody that targets SLAMF7, a glycoprotein highly expressed on multiple myeloma (MM) cells and natural killer cells. Elotuzumab exerts a dual effect, directly activating natural killer cells and mediating MM cell death via antibody-dependent cell-mediated cytotoxicity. In a 3-year follow-up of ELOQUENT-2 (NCT01239797), elotuzumab plus lenalidomide/dexamethasone (ELd) demonstrated a sustained 27% reduction in the risk of disease progression/death and an overall survival (OS) trend towards benefit compared with lenalidomide/dexamethasone (Ld) alone in patients with relapsed/refractory (RR) MM (Dimopoulos et al, ASH 2015).

Aims: To evaluate the long-term efficacy and safety of ELd following extended 4-year follow-up (median 46 months).

Methods: RRMM patients with 1-3 prior lines of therapy randomized 1:1 received ELd or Ld in 28-day cycles until disease progression/acceptable toxicity or consent withdrawal. Co-primary endpoints were progression-free survival (PFS) and overall response rate (ORR); OS was a secondary endpoint (analysis not prespecified for this data cut) and safety an exploratory endpoint. Written informed consent was obtained for all patients.

Results: In total, 646 RRMM patients were randomized: 321 to ELd and 325 to Ld. At 4-year follow-up (data cut-off: Oct 18, 2016), nearly twice as many patients remained on ELd therapy vs Ld (17% vs 9%). With the extended follow-up, ELd demonstrated a sustained relative improvement of 50% in PFS rates vs Ld (21% vs 14%) and maintained reduction in the risk of progression/death of 29% for ELd vs Ld (all randomized patients: HR 0.71; 95% CI 0.59, 0.86). Patients with every good partial response (VGPR) (ELd 112 [35%] vs 95 [29%]) had the greatest benefit in risk of progression/death (HR 0.65; 95% CI 0.46, 0.94). ORR was greater with ELd vs Ld (79% vs 66%) and the duration of response benefit was maintained over time (HR 0.77; 95% CI 0.62, 0.95). Early separation of the Kaplan–Meier survival curves, which remained consistently separated over time, supports a sustained OS benefit in favor of ELd vs Ld (Figure). Grade 3-4 adverse events in ≥5% of patients were generally comparable between ELd and Ld arms–vascular diseases (10% vs 8%; mostly venous-related), second primary malignancies (SPMs; 9% vs 6%) and cardiac disorders (5% vs 8%); the exception was a
S457
A PHASE IB STUDY OF ISATUXIMAB PLUS POMALIDOMIDE (POM) AND DEXAMETHASONE (DEX) IN RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM)

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Background: Isatuximab (ISA) is an anti-CD38 monoclonal antibody, which kills tumor cells via multiple mechanisms. Here, we report preliminary data from the dose-escalation cohorts, and the first 3 patients (pts) of the expansion cohort, of a Phase 1b study of ISA plus Pom/Dex in pts with RRMM (NCT02283775).

Aims: To evaluate combination therapy with ISA plus Pom/Dex in pts with RRMM.

Methods: Pts with RRMM (≥2 prior MM therapies, including lenalidomide and a proteasome inhibitor) were sequentially enrolled to ISA 5, 10, or 20mg/kg (4 weekly doses, then every 2 wks until disease progression) or intermittent toxicity with Pom 4mg (Days 1–21) and Dex 40mg (Days 1, 8, 15, and 22; 20mg if ≥75 yrs old), in 28-day cycles. An expansion cohort was initiated at ISA 10mg/kg (plus Pom/Dex) based on preliminary safety, efficacy, and PK data. Primary objective: to determine maximum tolerated dose (MTD). All patients were required to provide informed consent.

Results: 26 pts were analyzed (5mg/kg [n=8]; 10mg/kg [n=12]; 20mg/kg [n=6]), median age 65 (42–80) yrs. Median 4.0 (2.1–11) prior treatment regimens, with 20 (77%) pts refractory to prior immunomodulatory drug therapy. At data cut-off (Nov 8, 2016), median duration of ISA treatment was 19.0 wks and 16 pts off (Nov 8, 2016), median duration of ISA treatment was 19.0 wks and 16 pts remained on treatment. 12 pts at 10mg/kg discontinued therapy due to adverse events (AEs) (grade [Gr] 5 perforated bowel; Gr 3 infusion-associated reaction [IAR]). Dose-limiting toxicities reported in 1 pt at each dose level (Gr 4 neutropenia; Gr 4 neutropenic infection; Gr 3 confusion state), and MTD has not been reached. Most common TEAEs, besides IARs, were fatigue (62%), diarrhea (36.3%), rash (35%), and dyspnea (31%). Most frequent hematologic AEs (laboratory assessment) was neutropenia (Gr 3, 40%; Gr 4, 52%), Gr 3/4 thrombocytopenia was reported in 8 (32%) pts (Gr 3, 16%; Gr 4, 16%), IARs occurred in 12 (46%) pts (Gr ≥3 in 1 pt); only with 1st infusion in 9/12 pts. 16 (62%) pts were major causes of mortality in both arms; however, fewer deaths were reported with ELd vs Vd (165 vs 186).

Summary/Conclusions: At 4 yrs, ELd has the longest median follow-up of an immuno-oncology agent in MM. The data continue to show that adding eloctuzumab to Ld results in durable long-term responses, clinically relevant improvement in PFS, sustained reduction in risk of progression/death, and a survival trend in favor of ELd. Overall, these data continue to support the durability and efficacy of ELd. Updated safety and tolerability, including rate of SPMs, was consistent with previous findings despite longer exposure, with minimal incremental AEs compared with Ld therapy.

Study funding: BMS. Writing support: C Tomas, Caudex, funded by BMS.

S458
OVERALL SURVIVAL OF PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA TREATED WITH CARFILZOMIB AND DEXAMETHASONE VERSUS BORTEZOMIB AND DEXAMETHASONE IN THE RANDOMIZED PHASE 3 ENDEAVOR TRIAL

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Background: Daratumumab is a human monoclonal antibody targeting CD38 that induces deep and durable responses with significant clinical benefit and is well tolerated as monotherapy and in combination with established standard-of-care regimens in patients with RRMM.

Methods: Eligible patients with ≥2 prior lines of therapy were randomly assigned to 8 cycles (every 3 weeks) of Vd (1.3mg/m2) SC bortezomib on Days 1, 4, 8,
and 11; 20mg PO/IV dexamethasone on Days 1-2, 4-5, 8-9, and 11-12) with or without daratumumab (18mg/kg IV once weekly in Cycles 1-3, every 3 weeks for Cycles 4-8, then every 4 weeks until progression). Patients who were refractory to bortezomib were excluded. Progression-free survival (PFS) was the primary endpoint. Minimal residual disease (MRD) was assessed at suspected complete response (CR) and at 6 and 12 months after first dose at 3 sensitivity thresholds (10−6, 10−5, and 10−4) using the ClonoSEQ™ next-generation sequencing (NGS)-based assay (Adaptive Biotechnologies, Seattle, WA).

**Results:** A total of 498 patients were randomized with median (range) age of 64 (30-88) years. Patients received a median (range) of 2 (1-10) prior lines of therapy; 66% of patients previously received bortezomib, and 21% were refractory to lenalidomide in their last prior line of therapy. After median follow-up of 13.0 months, DVD significantly prolonged PFS compared with Vd alone (median: not reached vs 7.1 months; hazard ratio [HR], 0.33; 95% confidence interval [CI], 0.26-0.43; P<0.0001). Twelve-month PFS rates were 60% versus 22%, respectively. Significant PFS benefit was observed with DVD over Vd regardless of the number of prior lines of therapy, although the greatest benefit was seen in patients with 1 prior line of therapy (median: not reached vs 7.9 months; HR, 0.22; 95% CI, 0.14-0.34; P<0.0001). Overall response rate (ORR; 84% vs 63%) and rates of very good partial response (VGPR) or better (62% vs 29%) and CR or better (26% vs 10%) continued to be significantly higher with DVD compared with Vd (P<0.0001 for all). MRD-negative rates were more than 4 times higher at all 3 sensitivity thresholds with DVD versus Vd: 18.3% versus 3.6% at 10−6 (P<0.0001), 10.4% versus 2.4% at 10−5 (P<0.001), and 4.4% versus 0.8% at 10−4 (P<0.001). MRD-negative patients had prolonged PFS compared with MRD-positive patients at 10−4 sensitivity threshold (Figure). At the clinical cut-off date, 37 (15%) deaths in the DVD group and 58 (24%) in the Vd group have been observed (HR, 0.63; 95% CI, 0.42-0.96), and follow up is ongoing. Thrombocytopenia was the most common grade 3 or 4 treatment-emergent adverse event (45% with DVD vs 33% with Vd). No new safety signals were reported after median treatment duration of 11 months with daratumumab. Updated efficacy and safety data with longer follow up will be presented at the meeting.

**Summary/Conclusions:** Dvd is superior to Vd in terms of PFS, ORR, depth of response, and MRD-negative rates, with no new safety signals reported. These updated data further support the use of DVD as a standard of care in R/RMM, with the greatest benefit observed in patients with 1 prior line of therapy.

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**Aims:** The objectives of the study are to evaluate safety and preliminary efficacy of VEN with bortezomib and dexamethasone in relapsed/refractory (RR) MM.

**Methods:** Phase 1b study of patients (pts) with R/R MM who received daily VEN (50-1200mg for dose escalation cohorts; 800mg in safety expansion) with standard bortezomib (1.3mg/m2 SC) and dexamethasone (20mg PO).

**Results:** As of 19Aug2016, 86 pts were enrolled. Median age was 64 years; 9 (14%) pts had t(11;14), 5 (8%) had t(4;14), 15 (23%) had del(17p), and 30 (45%) had del(13q) abnormalities. Median number of prior therapies was 3 (range: 1-13), with 39% of pts refractory to prior bortezomib, 14% to carfilzomib, 53% to lenalidomide, and 21% to pomalidomide. Median time on study was 5.9 months (range: 0.3-28.9). Forty-six (70%) pts discontinued, with 36 due to disease progression (PD). Common AEs in ≥30% of pts were diarrhea (46%), constipation (41%), thrombocytopenia (39%), nausea (38%), peripheral neuropathy (33%), and insomnia (32%). Common grade 3/4 AEs in ≥10% of pts were thrombocytopenia (29%), anemia (15%) and neutropenia (14%). Serious AEs in ≥2 pts were febrile neutropenia, thrombocytopenia, cardiac failure, pyrexia, influenza, lower respiratory tract infection, pneumonia, sepsis, acute kidney injury, respiratory failure, embolism, and hypotension. Dose-limiting toxicities were grade 3 cardiac failure in the 300mg cohort (possibly related to dexamethasone) and grade 3 thrombocytopenia during the first cycle in the safety expansion. No events of laboratory or clinical TLS were reported. Four deaths were due to PD and 1 due to respiratory syncytial virus infection. Overall response rate (ORR) for all pts was 67% (44/66); 28 (42%) pts achieved very good partial response (VGPR) or better (3 stringent complete response [sCR], 10 CR, 15 VGPR). Pts non-refractory to prior proteasome inhibitors (PI) or immunomodulatory drugs (IMiDs) had higher ORR than refractory pts (PI, 92% vs 32%; IMiDs, 82% vs 57%). Among pts refractory to any 2 or more (n=15), 3 or more (n=7), or all 4 (n=4) prior therapies (bortezomib, carfilzomib, lenalidomide, pomalidomide), ORR was 40%, 43%, and 25%, respectively. Median time to progression (~10 vs 3 months) and duration of response (~10 vs 7 months) were longer for pts not refractory to any of these therapies versus refractory pts. ORR for pts with or without cytogenetic abnormalities, respectively, was as follows: 78% vs 65% for t(11;14), 60% vs 67% for t(4;14), 47% vs 73% for del(17p), and 63% vs 69% for del(13q).

**Summary/Conclusions:** VEN combined with bortezomib and dexamethasone has an acceptable safety profile with promising anti-myeloma activity, and the highest response rates were observed in R/R MM pts who were not refractory to PI or IMiDs. These data support the ongoing phase 3 trial with this regimen in R/R MM.
Improving prognostication and front-line therapy in chronic lymphocytic leukemia

S461

CYTOGENETIC COMPLEXITY IN CHRONIC LYMPHOCYTIC LEUKEMIA: DEFINITIONS, ASSOCIATIONS WITH OTHER BIOMARKERS AND CLINICAL IMPACT; A RETROSPECTIVE STUDY ON BEHALF OF ERIC


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Background: Recent evidence suggests that complex karyotype (CK) identified by chromosome banding analysis (CBA) may be a relevant biomarker for treatment decisions in CLL, especially regarding the response to signaling inhibitors. However, many challenges towards routine clinical application of CBA still need to be overcome.

Aims: Re-appraisal of definitions for CK in CLL and systematic investigation of clinicobiological associations and prognostic impact.

Methods: 3580 CLL and monoclonal B-cell lymphocytosis (MBL) patients (CLL=3322, 93% and MBL=258, 7%, respectively) were analysed with CGH-oligoDNA microarrays. CK was stratified into two groups with 3 low-CK (n=200, 52%), 4 (intermediate-CK) (n=822, 22%) and ≥5 (high-CK) (n=99, 25%) abnormalities. High-CK cases were stratified into those with 3 (low-CK, n=200, 52%), 4 (intermediate-CK, n=822, 22%) and ≥5 (high-CK, n=99, 25%) aberrations. High-CK cases were stratified into those with 3 (low-CK, n=200, 52%), 4 (intermediate-CK, n=822, 22%) and ≥5 (high-CK, n=99, 25%) aberrations.

Results: Following the current definition for CK i.e. ≥3 structural and/or numerical aberrations, 383/3580 cases (11%) displayed CK, with no difference in the detection rate between different cell stimulation protocols. CK was significantly associated (p<0.05) with male gender, advanced clinical stage (Binet B/C), TP53 mutation (79% and 88%, respectively). Main features of the studied cohort: median age: 65.6 years/males: 2252 (63%)/ Binet A/B/C: 2356/357/258 and no unfavorable cytogenetics (i.e. del(17p)/TP53 mutation and/or del(11q)).) Aims: The aim of this study was to analyse the outcome of M-CLL patients with no unfavorable cytogenetics CK according to the type of therapy.

Methods: We analysed 816 CLL patients from Sant Pau Hospital, Barcelona, Spain; Uppsala University Hospital, Sweden and IRCCS San Raffaele Scientific University, Milan, Italy for whom IGHV mutational status was available. Endpoints were OS and TFS.

Table 1.

Results: 488 patients had mutated IGHV genes (400 without unfavorable FISH cytogenetics; 26 had either del(11q) and/or del(17p), and in 62 cases FISH was not available) and 328 patients carried unmutated IGHV genes. The main clinical and biological characteristics at diagnosis are shown in Table 1. OS at 5 and 10 years was 93% (CI, 95-91) and 81% (CI, 85-77) for M-CLL cases and 78% (CI, 83-73) and 69% (CI, 72-64) for U-CLL cases (p<0.05). TFS at 5 and 10 years was 73% (CI, 69-77) and 50% (CI, 45-55) for M-CLL cases and 68% (CI, 63-73) and 48% (CI, 43-53) for U-CLL cases (p<0.05). After a median follow-up of 8 years (range, 1-26), 424 patients [161 M-CLL (136 without poor-prognostic FISH cytogenetics, 13 with either del(11q) and/or del(17p) and 12 cases in whom FISH information was not available) and 263 U-CLL (U-CLL) received therapy. Front-line treatment of front-line therapy (F/R) was associated with poor prognosis. In total, 315 (74%) patients were treated with purine analogues (PA)-based therapy (n=83), alkylating agents (n=212), anti-CD20 moAbs with PA or bendamustine (n=75), anti-CD20 moAbs with alkylating agents (n=21), BCR-signal inhibitors or BCL2 antiapoptotic agents (n=9), others (n=23), and unknown (n=1). The median follow-up after treatment was 7 years (range, 2-14).

Summary/Conclusions: CK defined by the presence of 3 numerical and/or structural abnormalities should not be axiomatically considered unfavorable in CLL, representing a heterogeneous group with variable clinical behavior. High-CK with ≥5 chromosomal aberrations emerges as prognostically adverse, independently of clinical stage, IG somatic hypermutation and TP53 status. Prospective clinical validation is warranted before finally incorporating high-CK in risk stratification in CLL.
median duration of response to first therapy was 42 months (range, 33-52) in M-CLL cases vs 24 months (range, 18-30) in U-CLL patients (p<0.001). 282 patients received a second line of therapy: PA-based therapy (n=95), alkylating agents (n=82), anti-CD20 MoAbs with PA or bendamustine (n=33), anti-CD20 MoAbs with alkylating agents (n=16), BCR-signal inhibitors or BCL2 antiprototic agents (n=12), others (n=39), and unknown (n=5). In 481 of 816 patients in whom detailed information on treatment regimens beyond second-line was available, 99 patients received a third-line treatment including PA-based therapy (n=15), alkylating regimens (n=20), anti-CD20 MoAbs with PA or bendamustine (n=15), anti-CD20 MoAbs with alkylating agents (n=8), BCR or BCL2 inhibitors (n=11), others (n=28) and unknown (n=2); 49 patients received four or more lines of therapy. In M-CLL patients without poor FISH cytogenetics (n=136) the type of therapy met patients’ outcome. Thus, the median survival was not reached in patients treated with CIT as first-line (i.e FCR, BR) as compared to 202 months in those not having received CIT (p=0.317). In contrast, in U-CLL patients the OS was highly dependent on the type of therapy. In detail, U-CLL patients who received anti-CD20 MoAbs with PA or bendamustine either as first line or subsequent lines (60 of 120 patients) showed significantly longer survival than those who did not receive these therapeutic regimens (median survival: 173 vs 103 months, p=0.001). On the contrary, in M-CLL cases no differences in survival were observed in those receiving anti-CD20 MoAbs with PA or bendamustine vs who did not (p=0.358).

Summary/Conclusions: This retrospective study suggests that OS of CLL patients with mutated IGHV genes and no unfavorable FISH cytogenetics do not depend on the type of therapy. This has important clinical implications and provides background for randomized studies aimed at identifying the optimal treatment strategy for this group of patients.

S463

IBRUTINIB PLUS FLUDARABINE, CYCLOPHOSPHAMIDE, AND OBINUTUZUMAB (GA101) IS CRITICALLY FOR PRIORITIZED TREATED PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) WITH MUTATED IGHV AND NON-DEL(17P)

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Background: Patients with mutated IGHV (IGHV-M) have favorable long-term outcomes (10-year PFS of >90%) after receiving first-line FCR. Aims: To develop an FC-based chemoimmunotherapy regimen of finite duration that included ibritinib and obinutuzumab. The intent was to limit FC to 3 courses, potentially reducing short- and long-term toxicity, while maintaining efficacy through the addition of ibritinib and a more potent antibody (obinutuzumab).

Methods: We designed an investigator-initiated phase II trial with ibritinib, fludarabine, cyclophosphamide, and obinutuzumab (IFCG) for previously untreated or untreated-resistant (R/R) cases of CLL with a median age ≥75 who were not randomized to the CLL2-BAG trial. This prospective, open-label, multicenter phase-II trial investigates a sequential treatment with a B debulking, followed by G and A as induction and maintenance therapy in an all-comer population of physically fit and unfit, treatment-naïve (TN) and relapsed/refractory (R/R) CLL pts. Methods: Pts with an absolute lymphocyte count (ALC) ≥25,000/μl and/or lymph nodes (LN) ≤5cm were to receive 2 cycles of B as debulking (70mg/m² d1& d2, q28 days), unless contraindicated. In the induction G (1000mg) was administered 3 times in cycle 1 (days 1/2, 8 & 15) and every 4 weeks in cycles 2-6. A was added in cycle 2 with a dose ramp-up (to 400mg daily) over 5 weeks and several safety precautions. In the maintenance therapy, daily intake of A was continued and G administered every 3 months until achievement of a MRD-negative complete response or for up to 24 months. The primary endpoint is the overall response rate (ORR) at the end of induction therapy; secondary endpoints include MRD evaluations, safety and survival parameters. This primary endpoint analysis is based on uncleaned data, the final analysis will be presented at the meeting.

Table 1.

<table>
<thead>
<tr>
<th>ORR</th>
<th>N=18</th>
<th>Marrow MRD PR</th>
<th>N=18</th>
<th>Marrow MRD PR</th>
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<td>11 (61)</td>
<td>7/11 (64) neg</td>
<td>5 (50)</td>
</tr>
<tr>
<td>PR</td>
<td>9 (50)</td>
<td>7 (43)</td>
<td>9/7 (71) neg</td>
<td>7 (35)</td>
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Summary/Conclusions: IFCG achieves high rate of MRD-neg remission after 3 courses. Pt enrollment continues, and updated results will be presented at the EHA meeting.
ed (table 1); with an ORR of 97%; at the end of induction, the primary endpoint was met. MRD negativity (<10−4) by flow cytometry in peripheral blood (pB) was achieved in 56 pts (89%); MRD assessment from bone marrow was available in 8 pts (4 TN and 4 R/R, among them 4 with a CR and 4 with a PR) and were all negative. As of January 9th, 2017, 35 serious adverse events (SAEs) were reported in 37 pts, including 69 SAEs (83%) related to study treatment. 66% of SAEs were CTCAE grades 3-4 and 1 had a fatal adverse event (sepsis at day 41 of induction cycle 1). Most SAEs occurred in the R/R cohort (61 SAEs, 74%) and during the induction phase (63 SAEs, 76%). Most common SAEs were infections (27 in 16 pts; including 13 CTCAE 3-5) and hematological disorders (18 in 10 pts; CTCAE 3-4), followed by infusion-related reactions (6 in 6 pts), laboratory TLS (5 in 5 pts) during debulking, 1 in induction cycle 1 with G2 in cycle 3 and 1 in cycle 4 with G and A) and ischemic coronary artery disorders (5 in 4 pts). No clinical TLS occurred.

Summary/Conclusions: With an ORR of 97% and a MRD negativity rate of 89%, front-line IDELA plus rituximab was shown to be highly active. The incidence of B-dubbing, followed by G and A was very efficacious in a heterogeneous study population and well tolerated except for 3 fatal septicemic in R/R pts. Efficacy, response duration, and overall survival were not analyzed due to early study termination. The reasons for discontinuation from study were death (10.8%), disease progression (14.7%), and other (11.8%). AEs of special interest included 6 on-study deaths, 3 associated with infection/fever (10.8%), diarrhea/colitis (11.8%). AEs of special interest included 6 on-study deaths, 3 associated with infection/fever (10.8%), diarrhea/colitis (11.8%). AEs of special interest included 6 on-study deaths, 3 associated with infection/fever (10.8%), diarrhea/colitis (11.8%). AEs of special interest included 6 on-study deaths, 3 associated with infection/fever (10.8%), diarrhea/colitis (11.8%).

Methods: Treatment-naïve pts with CLL and confirmed del(17p) were treated in a single arm study with R 375mg/m² IV weekly x 8 and IDELA 150mg PO BID continuously until disease progression or intolerability. Informed consent was obtained. The study was closed early due to safety reasons. Prior single arm studies have shown that front line use of IDELA may be associated with an increased frequency of transaminase elevations compared to relapsed pts. Aims: To describe: 1) the safety of IDELA plus rituximab in previously untreated CLL pts with del(17p) in this terminated study, and 2) the relation of key AEs and age.

Results: 102 pts (median age, 66; range, 37-86) were enrolled between Aug 2015 and Apr 2017. 56 pts (54.9%) achieved overall response (ORR) of 17p (37 pts with either del 17p or del 17q) or with either del 17q or del 17p, and 28 pts unsuitable for other therapies. Prior single arm studies have suggested that front line use of IDELA may be associated with an increased frequency of transaminase elevations compared to relapsed pts. Methods: Treatment-naive pts with CLL and confirmed del(17p) were treated in a single arm study with R 375mg/m² IV weekly x 8 and IDELA 150mg PO BID continuously until disease progression or intolerability. Informed consent was obtained. The study was closed early due to safety reasons. Prior single arm studies have shown that front line use of IDELA may be associated with an increased frequency of transaminase elevations compared to relapsed pts. Aims: To describe: 1) the safety of IDELA plus rituximab in previously untreated CLL pts with del(17p) in this terminated study, and 2) the relation of key AEs and age.

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Background: CC-122 is a cereblon modulating agent that degrades Aiolos and Ikaros, resulting in potent anti-lymphoma and immunomodulatory effects on T- and NK-cell function. Phase I clinical data revealed promising activity of CC-122 against follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL). Preclinical combination of CC-122 with obinutuzumab has shown synergism in FL and additive effects in DLBCL vs either single agent (Chiu, ASH 2015), supporting further study of this combination’s therapeutic potential. Aims: The current phase Ib study (EUDRACT 2014-003333-26; NCT02417285) evaluates the safety and efficacy of CC-122 and obinutuzumab in R/R patients with relapsed or refractory (R/R) B-cell non-Hodgkin lymphoma (NHL). Methods: Patients at study entry must have R/R CD20+ B-cell NHL after ≥1 prior regimens for FL/marginal zone lymphoma (MZL) and ≥2 regimens and/or ASCT for DLBCL. CC-122 was given orally (5 of 7 d) for 28-d cycles in escalating doses plus a fixed dose of intravenous obinutuzumab 1000mg on d2, 8, 15 of cycle (c1) and d1 of c2-c8, upon informed consent. CC-122 was continued until progressive disease (PD) or unacceptable toxicity. CC-122 active ingredient in capsule formulation (AIC) 1, 2, 3, and mg and CC-122 formulated capsules (F6) 3 and 4 mg were evaluated in separate cohorts. Primary endpoints included safety and tolerability, non-tolerated dose (NTD), and maximum tolerated dose (MTD). Response was assessed using the international Cheson 2007 criteria every 2 cycles to c6, every 3 cycles to c12, and every 6 cycles thereafter. Results: As of January 12, 2017, 34 R/R B-cell NHL patients with DLBCL (n=19), FL (n=15), or MZL (n=11) were enrolled. At study entry, median age was 60 y (26-81), most patients were male (68%), and Ann Arbor was extended stage III/IV in 76% of patients. Of the 18 DLBCL patients, 8 had transformed FL. Of the 16 FL/MZL patients, 44% relapsed in <12 months after first-line treatment. The median number of prior regimens was 4 (range, 1-11), and 13 (38%) patients had received prior SCT. One patient experienced a dose-limiting toxic effect (DLT) of grade 4 neutropenia (CC-122 dose level of AIC 3mg); no dose was yet an NTD. Median CC-122 duration was 22 wks (range, 3-71) equivalent to 6 cycles (range, 1-18). CC-122 dose reduction or temporary interruption occurred in 10 (29%) or 26 (76%) of patients, respectively, primarily due to adverse events (AEs). Most patients (96%) had ≥1 wk of interruption due to AEs. The most common (≥10%) grade 3/4 treatment-emergent AEs (TEAEs) were neutropenia (50%) and thrombocytopenia (21%). Fifteen patients (44%) had ≥1 serious TEAE, including 2 each of febrile neutropenia (related to CC-122), cytokine release syndrome (related to obinutuzumab), and pneumonia. Three deaths occurred during the study (2 PD; 1 AE-related). Overall response rate (ORR) was 59%, including 26% CR and 32% PR (Table 1). Median time to best response was 57 d, and median duration of response was not yet reached. In evaluable patients, 6-mo progression-free survival (PFS) was 63%. Table 1.

Summary/Conclusions: The combination of CC-122 and obinutuzumab was well tolerated and demonstrates promising response rates and durable remissions in R/R patients with B-cell NHL. CC-122 doses of ≥3mg and obinutuzumab doses of ≥3mg and obinutuzumab have shown best response rates to date. The study is ongoing to establish the phase II recommended dose.

S468

POLATUZUMAB VEDOTIN PLUS BENDAMUSTINE AND RITUXIMAB OR OBITINUTUMAB IN RELAPSED/REFRACTORY FOLLICULAR LYMPHOMA OR DIFFUSE LARGE B-CELL LYMPHOMA: UPDATED RESULTS OF A PHASE II STUDY

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1Memorial Sloan Kettering Cancer Center, New York, 2City of Hope, Duarte, University of Colorado, Denver, 3Department of Medicine, University of Birmingham, Birmingham, United States, 4Memorial Sloan Kettering Cancer Center, New York, 5Citi Bank

Background: Transplant ineligible patients (pts) with relapsed/refractory (R/R) FL or DLBCL have poor outcomes. Polatuzumab vedotin (pola) is an antibody drug conjugate that targets delivery of the microtubule inhibitor MMAE to cells expressing CD79b. Pola + rituximab (R) previously showed promising responses in R/R FL and DLBCL. Adding bendamustine (B) to polar-R and substituting obinutuzumab (G) for R could improve outcomes. We report updated results from the Phase Ib/2 (P1b/2) study evaluating pola + BR or BG in R/R FL and DLBCL and the expansion cohorts evaluating pola + BG in R/R FL and DLBCL (OmiciaTrials.gov NCT02257567).

Aims: The primary aim is to assess safety and tolerability of pola + BR/BG in R/R FL and DLBCL. Secondary aims include assessing safety and efficacy of pola + BG in an expansion cohort. Methods: All pts provided informed consent to participate in the study and were treated with pola (1.5mg/kg) + B (90mg/m²) and R (375mg/m²) or G (1000mg) every 28 days (FL) or 21 days (DLBCL) for 6 cycles. Responses were assessed by modified Lugano criteria after 3 cycles, end of treatment (tx), and every 6 months for 2 years during follow-up (fu).

Results: As of 14 Nov 2016, 65 pts were enrolled: 24 pts (12 FL, 12 DLBCL) in P1b and 41 pts (20 FL and 21 DLBCL) in P2. In safety evaluable pts, FL pts (N=32) were median age of 63 yr (37-86), 82% ECOG 0-1 and 8% Stage II/III, 2 (1-7) median lines of prior tx, 38% refractory to last tx, 13% prior transplant (BMT). DLBCL pts (N=32) were median age of 66 yr (30-86), 88% ECOG 0-1 and 13% ECOG 2, 39% IPI 3-5, 75% Stage II/III, 2 (1-7) median lines of prior tx. B was administered with pola in 50% of pts. 13 pts had ≥1 grade 3/4 adverse events (AEs) occurring in >20% of pts: fatigue (67%), nausea (54%), diarrhea (44%), vomiting (42%), pyrexia (39%) and constipation (39%). As expected, grade (Gr) 3/4 cytopenias were common: neutropenia (34% FL, 28% DLBCL), thrombocytopenia (16% FL, 13% DLBCL), anemia (6% FL, 12% DLBCL). Tx emergent neuropathy occurring in 19/64 (30%) of pts, with 1 Gr 3 event, and led to pola discontinuation in 1 pt, dose reduction in 2 pts, and interruption in 1 pt. In FL, 75% (24/32) had Gr 3/4 AEs and 41% (13/32) had serious AEs (SAEs). The only SAE occurring in ≥10% was infection (22%). The most common Gr 3/4 non-heme AEs were infection (16%) and hypokalemia (9%). AEs led to study tx discontinuation in 8 pts. B was stopped in 2 pts due to Gr 3 thrombocytopenia. Of 4 deaths; 2 were PD and 2 were Gr 5 AEs, 1 tx related (PML, 1 tx unrelated. In DLBCL, 88% (28/32) had Gr 3/4 AEs and 63% (20/32) had SAEs. Most Gr 3/4 non-heme AEs were febrile neutropenia (13%), fatigue (13%), and diarrhoea (13%). SAEs occurring in ≥20% of pts were infection (33%) and pyrexia (22%). AEs led to study tx interruption in 19 pts and discontinuation in 8 pts. There were 13 deaths: 9 PD, 4 AE (all unrelated to tx). Responses by modified Lugano 2014 criteria are shown in Table1. Median duration of response (DoR) for FL P1b pts was 16 months (mo)(median fu 14.5 mo). Median DoR for FL P2 (median fu 6.5 mo) and DLBCL P1b2 (median fu 13.7 mo P1b, 6.4 mo P2) have not been reached.

Summary/Conclusions: Updated evalution of pola + BR shows promising durable responses and an acceptable safety profile in heavily pre-treated R/R FL and DLBCL pts. Safety and efficacy data will be updated at the time of presentation.

S469

SINGLE AGENT ORAL SELINEXOR EXHIBITS DURABLE RESPONSES IN RELAPSED/REFRACTORY LARGE B-CELL LYMPHOMA (DLBCL) OF BOTH GCB AND NON-GCB SUBTYPES: THE PHASE 2B SADAL STUDY

M. Maerevoet1, J. Westin2, C. Thiebemann3, J. Zijlstra4, B.T. Hill5

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S469

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M. Maerevoet1, J. Westin2, C. Thiebemann3, J. Zijlstra4, B.T. Hill5
LYMPHOMA (R-R DLBCL)–A SINGLE-ARM PHASE II STUDY WITH RELAPSED OR REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA: MOR208 COMBINED WITH LENALIDOMIDE (LEN) IN PATIENTS

Background: Patients (pts) with persistent DLBCL after two or more lines of therapy have limited effective treatment options. The nuclear export protein exportin 1 (XPO1) is upregulated in hematologic malignancies, including DLBCL, and has pleiotropic effects on tumorigenesis including functional downregulation of tumor suppressor proteins (TSPs) and increased export and translation of mRNAs for oncoproteins c-Myc and key survival proteins such as Bcl-2. Selinexor (SEL), an oral XPO1 inhibitor, causes sequestration of TSPs including p53, p21, and IκBα, the latter of which serves to suppress NF-κB activity.

Methods: Pts with R/R DLBCL were randomized to 60 or 100mg of SEL twice weekly (8 doses) per 28-day cycle. Pts were also stratified by DLBCL subtype (GCB or non-GCB). The primary objectives are to determine the ORR and evaluate the safety of 60 vs 100mg doses. Disease response was assessed by an Independent Central Radiological Review (ICRR), using the Lugano Classification (Cheson, 2014).

Results: 72 pts were enrolled: 37 pts on 60mg (24 M/13 F, median age 71 yrs) and 35 pts on 100mg (23 M/12 F, median age 68 yrs). Both groups had a median of 3 prior treatment regimens. The most common related adverse effects (AEs) across both dosing groups (Grade 1/2) were: fatigue (47%), nausea (46%), anorexia (42%), and vomiting (33%). Common Grade 3/4 AEs were: thrombocytopenia (39%), fatigue (18%), neutropenia (18%), and anemia (13%). These were managed with dose interruption/reduction, platelet stimulators, and/or standard supportive care. Grade 3/4 fatigue (26% v 11%) and thrombocytopenia (39/26), fatigue (18%), neutropenia (18%), and anemia (13%).

Summary/Conclusions:

SEL monotherapy shows activity in pts with R/R DLBCL with a median of 3 prior treatment regimens. Median age was 74 years (range 47–82); 45% of patients received ≥2 prior lines of therapy; 23% had rituximab refractory disease; 74% had Ann Arbor stage ≥III disease; 65% had elevated lactate dehydrogenase level, and 52% had a poor revised International Prognostic Index (3–5). The most common treatment-emergent adverse events (any grade) were neutropenia (39/26), anemia (23/0)/thrombocytopenia (16/6), infections (26/10)/diarrhea (13/0), pyrexia (13/0), and rashes (13/6). Of 26 response evaluable patients (median follow-up 3.3 months), ORR (investigator assessed) was 58% (15 patients), with 7 (27%) complete responses. Median time to response was 1.8 months.

Table 1. Independent Central Radiological Review-Best Response.

<table>
<thead>
<tr>
<th>Category</th>
<th>N</th>
<th>ORR (%)</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>SD (%)</th>
<th>DCR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Doses</td>
<td>66</td>
<td>18 (27.3%)</td>
<td>7 (11.2%)</td>
<td>11 (16.7%)</td>
<td>9 (13.6%)</td>
<td>27 (40.6%)</td>
</tr>
<tr>
<td>60 mg</td>
<td>32</td>
<td>9 (28.1%)</td>
<td>4 (12.5%)</td>
<td>5 (15.6%)</td>
<td>3 (9.4%)</td>
<td>20 (62.5%)</td>
</tr>
<tr>
<td>100 mg</td>
<td>34</td>
<td>9 (26.5%)</td>
<td>3 (8.8%)</td>
<td>6 (17.6%)</td>
<td>0 (0%)</td>
<td>20 (58.8%)</td>
</tr>
<tr>
<td>GS+</td>
<td>5</td>
<td>2 (40.0%)</td>
<td>1 (20.0%)</td>
<td>2 (40.0%)</td>
<td>0 (0%)</td>
<td>100.0%</td>
</tr>
<tr>
<td>GS-</td>
<td>61</td>
<td>16 (26.2%)</td>
<td>7 (11.5%)</td>
<td>12 (19.7%)</td>
<td>20 (32.8%)</td>
<td>63 (102.6%)</td>
</tr>
<tr>
<td>Non-GC Subtype</td>
<td>51</td>
<td>15 (29.4%)</td>
<td>4 (7.8%)</td>
<td>15 (29.4%)</td>
<td>12 (23.5%)</td>
<td>44 (86.3%)</td>
</tr>
</tbody>
</table>

Summary/Conclusions: SEL monotherapy shows activity in pts with R/R DLBCL including in pts with GCB subtype. 60mg SEL twice weekly was more tolerable than 100mg twice weekly, with fewer interruptions due to toxicity. Objective responses to SEL were durable at 60mg BIW, suggesting these responses were associated with clinical benefit.
Targeted treatment of AML

S471
ENASIDENIB (AG-221) IN MUTANT-IDH2 RELAPSED OR REFRactory ACUTE MYELOID LEUKEMIA (R/R AML): RESULTS OF A PHASE 1 DOSE-ESCALATION AND EXPANSION STUDY

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Background: Recurrent mutations in isocitrate dehydrogenase 2 (mIDH2) occur in ~12% of AML patients (pts), mIDH2 proteins synthesize an oncometabolite, 2-hydroxyglutarate (2HG), causing DNA and histone hypermethylation and blocked myeloid differentiation. Enasidenib (AG-221) is an oral, selective, small-molecule inhibitor of mIDH2 proteins. Differentiation of myeloblasts, not cytotoxicity, appears to drive the clinical efficacy of enasidenib. In preclinical studies, bone marrow blasts from pts with mIDH2-IDH2-R140 erythroleukemia (TF-1) cells treated with enasidenib for 7 days in vitro occur in ~12% of AML patients (pts), mIDH2 proteins synthesize an oncometabolite, 2-hydroxyglutarate (2HG), causing DNA and histone hypermethylation and blocked myeloid differentiation. Enasidenib (AG-221) is an oral, selective, small-molecule inhibitor of mIDH2 proteins. Differentiation of myeloblasts, not cytotoxicity, appears to drive the clinical efficacy of enasidenib. In preclinical studies, bone marrow blasts from pts with mIDH2-IDH2-R140 erythroleukemia (TF-1) cells treated with enasidenib ex vivo were shown to produce mature, fully functioning neutrophils with conserved mIDH2 allele frequency, indicating differentiation of mature cells from the mIDH2 blasts (Yen et al, Cancer Discov, 2017). Additionally, no apoptosis was observed in mIDH2-R140 erythroleukemia (TF-1) cells treated with enasidenib for 7 days in vitro.

Table 1.

<table>
<thead>
<tr>
<th>Table: Patient characteristics, response rates, and response time to treatment.</th>
<th>Relapsed or refractory AML</th>
<th>All doses (n=176)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median age (years)</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Clinical presentation</td>
<td>102 (58%)</td>
<td>55 (31%)</td>
</tr>
<tr>
<td><strong>Response rates</strong></td>
<td></td>
<td></td>
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<tr>
<td>Complete response (CR)</td>
<td>5 (29%)</td>
<td>5 (29%)</td>
</tr>
<tr>
<td>CR with incomplete remission</td>
<td>14 (82%)</td>
<td>14 (82%)</td>
</tr>
<tr>
<td><strong>Response time to treatment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to first treatment</td>
<td>10.7 days</td>
<td>10.7 days</td>
</tr>
<tr>
<td><strong>Results</strong></td>
<td>Relapsed or refractory AML</td>
<td>All doses (n=176)</td>
</tr>
<tr>
<td>Overall response rate (ORR) including CR</td>
<td>40.3%</td>
<td>40.3%</td>
</tr>
</tbody>
</table>
| **Summary/Conclusions:** Enasidenib was well tolerated, induced CRs in R/R AML pts, and was associated with OS of ~9 mos in pts who had failed prior AML Tx. A randomized phase 3 study of enasidenib vs conventional care in older pts with late-stage R/R AML is ongoing (NCT02577406).

S472
SAFETY AND EFFICACY OF VENETOCLAX (VEN) IN COMBINATION WITH DECITABINE OR AZACITIDINE IN TREATMENT-NAIVE, ELDERLY PATIENTS (≥65 YEARS) WITH ACUTE MYELOID LEUKEMIA (AML)

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Background: Newly diagnosed patients (pts) with AML aged ≥65 years and ineligible for standard induction therapy have limited treatment options, and low overall survival. VEN is an orally bioavailable, selective BCL-2 inhibitor that has displayed single-agent activity in pts with relapsed/refractory AML. VEN at escalating doses combined with hypomethylating agents (HMAs) has demonstrated antileukemic activity, with an overall response rate (ORR) including complete remission [CR], and CR with incomplete marrow recovery of 60%. Combining VEN with HMAs, such as decitabine (DEC) or azacitidine (AZA), may provide a novel low-intensity approach for treating AML. Preliminary results from the expansion stage of a phase 1b trial comparing 2 doses of VEN plus either DEC or AZA (NCT02203773) are reported.

Aims: To evaluate the safety and efficacy of VEN at 400-mg vs 800-mg doses plus DEC or AZA.

Methods: This open-label, nonrandomized, two-stage phase 1b study evaluated the safety and efficacy of VEN plus DEC or AZA in treatment-naive pts ≥65 years old with de novo AML. Eligibility excluded: ECOG PS ≥2; ineligible for standard induction therapy; intermediate- or poor-risk karyotype. Pts received DEc (Arm D, 20mg/m²/day [d]; intravenous [IV]) on d1–5, or AZA (Arm E, 75mg/m²/d; subcutaneous or IV) on d1–7 of each 28-d cycle (C) in combination with once-daily VEN. The dose-escalation stage consisted of 2 VEN dose cohorts (continuous 400-mg and interrupted 800-mg dosing) in each arm (D1, D2, E1, and E2, respectively) to determine optimal dose. Tumor lysis syndrome (TLS) prophylaxis was administered in C1 to all pts during VEN dose- ramp-up until final dose was reached. All pts provided informed consent.

Results: In all, 239 pts received enasidenib. Median age was 70 yrs. In the dose-escalation phase (n=113), pts received daily enasidenib doses of 50–650mg. The MTD was not reached. Median 2HG reductions from baseline at cycle 2 day 1 were 92%, 90%, and 93% for pts receiving <100mg, 100mg, and >100mg/day, respectively. Enasidenib 100mg QD was chosen for the expansion phase (n=126) based on PK/PD profiles and demonstrated efficacy. Median number of enasidenib cycles was 5 (range 1–25). Grade ≥3 investigational adverse events in R/R AML pts was 40.3%, including 34 pts (19.3%) who attained CR. Median time to 1st response was 1.9 months (mos); 87.3% of responding pts attained a 1st response by cycle 5. Median response duration was 5.8 mos. Of pts who achieved CR, 7 pts (21%) did so by cycle 3, 23 (88%) by cycle 5, and 29 (82%) by cycle 7. Median duration of CR was 8.8 mos. ORR with enasidenib 100mg/day was 38.5% (Table). Seventeen pts (11%) proceeded to stem cell transplant. Response was associated with cellular differentiation, typically with no evidence of aplasia. Median overall survival (OS) of R/R AML pts was 9.3 mos. For pts who attained CR, OS was 19.7 mos. Pts who had received ≥2 prior AML Tx had a median OS of 8.0 mos.

Summary/Conclusions: Enasidenib was well tolerated, induced CRs in R/R AML pts, and was associated with OS of ~9 mos in pts who had failed prior AML Tx. A randomized phase 3 study of enasidenib vs conventional care in older pts with late-stage R/R AML is ongoing (NCT02577406).

haematologica | 2017; 102(s2) | 175

Madrid, Spain, June 22 – 25, 2017
Results: As of 13/09/16, 100 pts were enrolled in the expansion stage: 25 pts in each arm. Overall, 61% pts were males; 59% had ECOG PS 1 and 15% ECOG PS 2; mean age was 73.9 (range 65–86); 53% had adverse karyotype; and 22% had secondary AML. Median time on study was 6 (4.9), 6 (0.2–9), 5 (0.5–9), and 4 (1–8) mo for arms D1, D2, E1, and E2, respectively. The incidence of adverse events (AEs) was generally comparable between the 4 arms. Overall, the most common treatment-emergent AEs (TEAEs; in ≥30% of pts) were nausea (59%), diarrhea (42%), febrile neutropenia (FN; 41%), constipation (39%), fatigue, and decreased white blood cell count (31% each). The most frequent grade 3/4 TEAE and serious AE was FN (41%) and 29%, respectively. No TLS was observed. Overall, 29 pts discontinued the study for ≥1 reason, including progressive disease (PD) per protocol (n=10), “other” (n=10; 9/10 proceeded to stem cell transplantation) and AEs not related to progression (n=10). A total of 16 deaths occurred; 12 pts died within 30 d of initiating VEN and HMA due to AEs (n=12) and PD (n=1). The ORR was 68%, with rates of 76% (19/25), 71% (16/23), 68% (16/24), and 65% (15/23) observed in arms D1, D2, E1, and E2, respectively. The Kaplan-Meier survival curve for all pts with a median follow-up time of 5.4 mo is shown.

Summary/Conclusions: Overall, the safety profile was favorable when combining VEN at either dose with DEC or AZA in treatment-naive elderly AML pts. Promising activity with high ORRs was observed at the lower 400-mg VEN dose in both HMA arms. A Phase 3 study of VEN plus AZA is planned.

Aims: Evaluate the safety and efficacy of VEN+LDAC in older pts with untreated AML.

Methods: In this open-label phase 1/2 study, pts ≥65 years with untreated AML, ineligible for standard induction chemotherapy, with an ECOG performance status of 0–2 received oral VEN QD on days (d) 1–28 and subcutaneous LDAC 20mg/m² QD on d 1–10 of each 28-d cycle. VEN target dose evaluation followed a 3+3 design, ranging from 600–800mg; 18 pts were enrolled and the RP2D was established as 600mg. Safety and efficacy of VEN at RP2D were evaluated in the expansion phase. All pts were hospitalized and received prophylaxis before a dose ramp-up of VEN during cycle 1 to mitigate the risk of tumor lysis syndrome (TLS). Adverse events (AEs) were graded by NCI CTCAE V4.0. Pts enrolled as of May 2016 are included in this analysis; data cutoff was August 2016. All pts provided informed consent.

Results: In total, 61 pts, including 8 from phase 1, were treated at the RP2D of 600mg (median age 74 years; ECOG 1–2; 70% adverse karyotypes; 33% secondary AML; 44% prior hypomethylating agent [HMA] 28%). AEs (all grade; severe grade) including cytopenias were nausea (72%), hypokalemia (46%), diarrhea (44%), fatigue (43%), and decreased appetite (41%). Grade 3/4 AEs (≥10% pts) were febrile neutropenia (34%), hypokalemia (15%), hypophosphatemia (13%), and hypertension (10%). No pts had clinical TLS; 1 pt had laboratory TLS, which was managed. The 30-d and 60-d mortality rates were 3% and 15%, respectively. The CR/CRi rate was 54% (33/61; 21% CR and 33% CRi). The overall response rate (ORR; CR+CRi+partial remission) was 61% (37/61). VEN+LDAC was shown to be active across a wide range of cytogenetic mutations and pt profiles (ORR: 70% in pts ≥75 years; 52% in second-line AML). 47% in pts with adverse karyotypes; 53% in pts with prior HMA). Among response-evaluable pts, those achieving an objective response have longer survival than pts who do not achieve an objective response (Figure 1).

Figure 1. 

Summary/Conclusions: VEN (RP2D 600mg) and LDAC exhibited an acceptable safety profile and durable efficacy in pts aged ≥65 years with untreated AML who are ineligible for or unable to receive intensive induction chemotherapy. ORR highly correlated with overall survival, with better survival observed in responders compared with nonresponders. A planned phase 3 randomized trial has commenced.

Aims: To assess the best response to Aza+Nivo at the end of 3 courses of combination therapy.

Methods: Pts were eligible if they had AML and failed prior therapy, had adequate performance status (ECOG ≤2), and organ function. The first six pts
received AZA 75mg/m² Days 1-7 with nivolumab 3mg/kg on Day 1 and 14. Courses were repeated every 4-5 weeks indefinitely. Only one of six pts had a dose limiting toxicity (grade 3 pneumonitis) and this dose was RP2D. 60 additional pts have been treated at the RP2D.

Results: 66 pts with a median age of 71 years (range, 44-90), secondary AML (39%), poor risk cytogenetics (35%), median number of prior regimens 2 (range, 1-7) have been enrolled. All 66 pts had baseline next generation sequencing: TP53 (n=11), DNMT3A (n=12), ASXL1 (n=10), TET2 (N=9), and RAS (n=9), IDH2 (n=9), IDH1 (n=6), CEBPA (n=7). 63 pts are evaluable for response: 14 (22%) achieved complete remission (CR)/complete remission with insufficient recovery of counts (CRi) (3 CR, 11 CRi), 7 (11%) had hematologic improvement (HI) (3 HI, 4 HI+), and NR was 5 (8%) had stable disease >6 months, and 24 (38%) had progression. 3 pts are too early for response assessment (<3 courses). The median number of courses to CR/CRi/HI was 2 (range, 1-4+). The median OS among the CR/CRi/HI patients was 15.3 months (range, 2.29-17.45+), and NR was 5.0 months (range, 0.29-16.16). The 4- and 8-week mortality were 5% and 11%, respectively. The median OS for the 63 evaluable pts on Aza+Nivo compares favorably to historical median OS with AZA-based salvage protocols in similar pts treated at MDACC (P=0.10) (Fig 1A and Fig 1B). Grade 3/4 and Grade 2 immune toxicities were observed in 8 (12%) and 7 (11%) pts, respectively. The most common Grade 3/4 AEs on treatment included pneumonitis, colitis, nephritis, skin rash, and hypophysitis. One pt died from grade 4 pneumonitis/epiglottitis. In the remaining 14 cases the toxicities responded rapidly to steroids and 13 of these pts were successfully rechallenged with nivolumab. Time to onset of toxicities ranged from 4 days to 3.5 months. Multicolor flow-cytometry studies and Mass-cytometry (CyTOF) studies are conducted by the Immunotherapy Platform on baseline and on-treatment BM aspirate (end of cycle 1, 2, 4, 8). Baseline and end of cycle (EOC) 1 and 2 BM was evaluated in 6 responders and 19 non-responders. Pts who achieved a response had a baseline higher live total CD3+ cells and BM CD3+ cells, with increased ICOS (activation) marker on BM CD4-effector cells at EOC 1 and EOC 2 as compared to those who had no response. The CTLA4 on CD8 T-cells went up in both responders and non-responders after PD1 based therapy.

Summary/Conclusions: Full dose AZA and nivolumab are tolerable and produce an encouraging response rate with durable responses in relapsed AML with poor risk features. Immune mediated toxicities occur and may be adequately managed with early recognition and systemic steroids. Up-regulation of CTLA4 may be a mechanism of resistance to PD1 based therapies in AML and suggest role for combination therapy.

S475

QUIZARTINIB AND BRIDGE TO TRANSPLANT IN FLT3-ITD AML PATIENTS AFTER FAILURE OF SALVAGE CHEMOTHERAPY: A HISTORICAL COMPARISON WITH UK NATIONAL CANCER RESEARCH INSTITUTE (NCRI) DATA

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Background: The presence of a FMS-like tyrosine kinase 3 (FLT3) Internal Tandem Duplication (ITD) mutation in pts with AML is associated with an increased early relapse rate and a dismal prognosis. Quizartinib is a potent, selective FLT3 inhibitor that conferred a median overall survival (mOS) of 23 weeks and remission rate of 46% in a single-arm phase 2 study (AC220-002) in pts with AML with a FLT3-ITD mutation who were relapsed or refractory (R/R) to second line therapy. (Levis, et al, ASH 2012) As context, a study of AML pts, regardless of FLT3 mutation status, receiving second-salvage therapy achieved a mOS of only 1.5 months. (Giles F, et al. Cancer 104 (3), 2005). Such poor-risk pts may benefit from a stem cell transplant (SCT), if available.

Aims: The primary aim was to compare SCT rates and outcomes of pts on quizartinib from an exploratory selected cohort in the AC220-002 study with those from a historical cohort of 1388 AML pts with confirmed FLT3-ITD mutations in the UK NCRI database.

Methods: Within AC220-002, 58 pts with a FLT3-ITD mutation were identified who had received intensive chemotherapy, and were relapsed (n=53), or refractory (n=5) to salvage therapy prior to entry. Applying the same entry criteria to the NCRI database, we identified 118 pts who received only recognized chemotherapy regimens prior to eligibility (relapsed n=99; refractory n=19). To avoid biases where those dying early would predominantly contribute to the NCRI group (reflecting that pts in AC220-002 had to be fit enough to be enrolled), pts in this cohort entered analysis 14 days following being identified as R/R. Multivariable Cox/logistic regression was used to compare remission rates and survival stratified for known prognostic factors. A landmark analysis excluding deaths before day 90 (allowing for those too unfit for SCT) was performed on the pooled sample (n=176) of the AC220-002 and NCRI cohorts to compare survival between transplanted and non-transplanted pts.

Results: Overall, quizartinib-treated pts had significantly greater remission rates, consisting mainly of complete remission without normal blood counts (CRI), vs NCRI pts (40% vs 3%, adjusted OR 0.05 (0.01-0.21), p<0.0001) and improved mOS (140d vs 54d, adjusted HR 0.38 (0.25-0.58) p<0.0001). A greater proportion of pts in AC220-002 proceeded to SCT: 23/54 (40%) vs 9/118 (8%). Comparing survival in SCT vs no-SCT in a landmark analysis, 18-month survival was significantly greater in the SCT group (29% vs 7%, adjusted HR 0.36 (0.20-0.65) p<0.0005). Significance persisted in sensitivity analyses with the landmark set at 120 or 150 days indicating an association between long-term survival and SCT. A similar analysis in an unmatched cohort consisting of SCT-naive pts in first relapse also found better survival for SCT vs no-SCT, confirming a potential benefit of SCT in this poor risk population.

Summary/Conclusions: When compared to a large historical cohort, quizartinib was associated with greater remission rates and opportunity to receive SCT in pts who relapsed after salvage therapy. While varying practice patterns and patient factors obviously influence treatment choices and outcomes, pts with AML with FLT3-ITD mutation appeared to benefit with longer survival observed with SCT. This data suggests quizartinib may show promise in potentially improving long-term survival by bridging patients to SCT.
S476

GLOBAL REGISTRATION TRIAL OF EFFICACY AND SAFETY OF CTL019 IN PEDIATRIC AND YOUNG ADULT PATIENTS WITH RELAPSED/REFRACTORY (R/R) ACUTE LYMPHOBластIC LEUKEMIA (ALL): UPDATE TO THE INTERIM ANALYSIS

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Background: The CD19-targeted chimeric antigen receptor (CAR) T-cell therapy CTL019, an investigational therapy that reprograms cytotoxic T cells to eliminate target cells, resulted in high response rates and a manageable safety profile in pediatric/young adult patients (pts) with R/R B-cell ALL in a single-center trial. Aims: We report an updated interim analysis from the first multicenter global pivotal phase 2 trial of CTL019 in pediatric/young adult pts with CD19+ R/R B-cell ALL with ≥5% bone marrow lymphoblasts by morphologic morphology, CTL019 was manufactured from leukapheresis of autologous peripheral blood T cells at a centralized manufacturing facility. The primary endpoint was overall remission rate (complete remission [CR] + CR with incomplete blood count recovery [CRi]) within 3 mo. Secondary endpoints included duration of remission (DOR), overall survival, safety, and cellular kinetics. Results: As of November 2016, 88 pts were enrolled. There were 7 (8%) manufacturing failures, 9 (10%) pts were not infused due to death or adverse events (AEs), and 4 pts (5%) were pending infusion at the time of data cutoff. Following lymphodepleting chemotherapy in most pts (fludarabine/cyclophosphamide [n=64] or other [n=1]), 68 pts were infused with a single dose of CTL019 (median dose, 3.04×10^6/kg [range, 0.25-6.4×10^6/kg]) transduced CTL019 cells/kg), with a median study follow-up of 6.4 mo. Median age was 12 y (range, 3-23 y); 59% of pts had prior allogeneic stem cell transplantation (alloSCT). Five infused patients had not reached 3 mo of follow-up; among 63 evaluable pts, 52 (83% [95% CI, 71%-91%]) achieved CR/CRi within 3 mo of CTL019 infusion, all of whom had minimal residual disease (MRD) negativity. The relapse-free probability at 6 mo after remission onset was 75% (95% CI, 57%-87%; median DOR not reached). The probability of survival was 89% (95% CI, 77%-94%) at 6 mo and 79% (95% CI, 63%-89%) at 12 mo. Seven pts (13% of responders) proceeded to alloSCT within 6 months while in remission. Cytokine release syndrome (CRS) was graded using the Common Terminology Criteria for Adverse Events (CTCAE) v4.0 criteria. No grade 4 CRS occurred in 78% of pts (21% grade 3; 27% grade 4); no CRS-associated deaths occurred. 38% of pts received tocilizumab for treatment of CRS with or without other anti-cytokine therapy. Most pts (82% [n=54]) had grade 3/4 nonhematologic AEs (~15%) other than CRS were hypotension (22%), hyponatremia (18%), and increased transaminase transferase (ALT/AST) (18.2%); 6% of pts experienced grade 3 neurocytotoxic AEs, with no grade 4 events and no cerebral edema reported. Grade 3/4 neutropenia with high (>38.3°C) fever occurred in 60% of pts. 2 pts died within 30 days of infusion (ALL progression, n=1; cerebral hemorrhage, n=1), and 9 pts died >30 days after infusion (ALL relapse/progression, n=6; HHV-6 encephalitis, pneumonia, systemic mycosis, n=1 each). CTL019 expansion in vivo correlated with CRS severity, and persistence of CTL019 along with B-cell aplasia in peripheral blood was observed for ≥1 year in some responders. Summary/Conclusions: The ELIANA study confirmed the efficacy of a single dose of CTL019, without additional therapy, observed in a previous interim analysis and a prior single-center CTL019 trial. AEs were effectively and reproducibly managed globally by appropriately trained personnel at study sites.

S477

CTL019 CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS IN PEDIATRIC PATIENTS (PTS) WITH RELAPSED OR REFRACTORY (R/R) ACUTE LYMPHOBластIC LEUKEMIA (ALL)

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Background: CTL019 is an investigational therapy whereby autologous T cells are genetically engineered with a chimeric antigen receptor (CAR) to identify and eliminate CD19-expressing malignant B cells. Data from 2 phase 2 studies (ELIANA; NCT02435849 and ENSIGN; NCT02228096) in pediatric and young adult R/R B-cell ALL were pooled to evaluate cellular kinetics of CTL019. Aims: We report cellular kinetics, humoral immunogenicity, AUC-28d (exposure)-response analysis and impact of intrinsic/extrinsic and manufacturing factors on CTL019 expansion. Methods: Cellular kinetic parameters of CTL019 post-infusion were derived using traditional pharmacokinetic principles and reported by response category (complete response [CR]/CR with incomplete blood count recovery [CRi] vs no response [NR]) using 2 assays of peripheral blood cells: qPCR and flow cytometry. AUC-28d-response relationships were evaluated by logistic regression. Relationships between manufacturing specifications, therapies for cytokine release syndrome (CRS) management, and anti-CAR19 antibodies on cellular kinetics explored using summary statistics and graphical- and model-based analyses.

Figure 1.

Results: Data from 79 pts (ELIANA, n=50; ENSIGN, n=29) were pooled for analysis. Using qPCR, pts with CR/CRi (n=62) had ≥2-fold higher CTL019 expansion than pts with NR (n=7) (Cmax, 73.5% higher geometric [geo] mean; AUC0-28d, 104% higher geo mean; Table 1). Pts with NR had delayed Tmax compared with pts with CR/CRi (20 vs 10 days). Intrinsic pt factors including baseline cytogenetics, disease characteristics, and disease status did not appear to affect Cmax or AUC-28d with the exception that pts with a higher tumor burden at enrollment generally had higher expansion, based on box plots of summary statistics. Extrinsic factors (prior lines of therapy, stem cell transplant) and parameters related to the manufactured product (% T cells, transduction efficiency, cell viability, total cell count), did not appear to impact cellular kinetics, based on graphical analysis. AUC-28d increased with pres-
ence and severity of CRS. Pts who received anti-cytokine agents for grade 3/4 CRS also had higher expansion, CR/CRi pts treated with tocilizumab and steroids (n=17) had 89% higher AUC0-28d vs CR pts who did not receive tocilizumab and steroids (n=45). Experience is limited in NR pts with (n=4) and without (n=4) tocilizumab. Moderate correlation was observed between trans-gene levels and CAR surface expression in peripheral blood (r2=0.592) by qPCR and flow cytometry, respectively, when matched by time points from the cellular kinetic profile. Slower B-cell recovery was observed in pts with AUC0-28d above the median. Post-dose anti-CAR19 antibody responses were determined from the fold change of anti-CAR19 antibodies above the baseline pre-dose value. Pts with treatment-induced or boosted anti-CAR19 antibody responses generally had lower expansion, based on box plots, compared with pts with treatment-unaffected anti-CAR19 antibody responses, although AUC0-28d was variable. The boosted levels of anti-CAR19 did not impact clinical response or relapse.

Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median (95% CI)</th>
<th>S1 (n=179)</th>
<th>S2+ (n=134)</th>
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<tr>
<td>AUC0-28d</td>
<td>5.1 (3.2, 7.1)</td>
<td>5.1 (3.2, 7.1)</td>
<td>5.1 (3.2, 7.1)</td>
</tr>
<tr>
<td>CR/CRi</td>
<td>4.8 (2.8, 6.8)</td>
<td>4.8 (2.8, 6.8)</td>
<td>4.8 (2.8, 6.8)</td>
</tr>
</tbody>
</table>

Summary/Conclusions: There was increased expansion of CTL019 in pts with higher tumor burden at enrollment, which correlated with higher CRS grade. There was no relationship between dose and expansion, supporting the wide dose range used. Expansion was not attenuated by tocilizumab, steroids, indicating therapies for CRS do not abrogate CTL019 proliferation. Cellular kinetics are important to understand the determinants of tumor response with CAR T-cell therapy.

S478

BLINATUMOMAB VS SOC CHEMOTHERAPY IN FIRST SALVAGE COMPARED WITH SECOND OR GREATER SALVAGE IN A PHASE 3 STUDY

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Background: Adults with B-cell precursor (BCP) acute lymphoblastic leukemia (ALL) often relapse following standard induction/consolidation chemotherapy (CR/CRI) and second and subsequent CTX salvage regimens (S2+) is poor compared with first salvage (S1) or frontline therapy. Blinatumomab links cytotoxic CD3-positive T cells and CD19-positive B cells to induce tumor cell lysis. In a randomized phase 3 trial of blinatumomab vs investigator’s choice of 4 standard of care CTX (SOC) regimens, median OS was 7.7 months in the blinatumomab group vs 4.0 months with SOC (Kantarjian H, et al., NEJM 2017). Here, we evaluate outcomes by salvage status for patients in this study (NCT02013167).

Aims: To evaluate responses to blinatumomab vs SOC in patients with relapsed/refractory ALL by prior salvage therapy status.

Methods: Patients with relapsed/refractory (R/R) BCP-ALL in this international multicenter trial were randomized 2:1 to blinatumomab (n=271) or SOC (n=134). For this analysis, salvage status was adjudicated separately from prior randomization. Blinatumomab was given by continuous IV infusion (9 µg/d in week 1 of cycle 1, then 28 µg/d in cycles of 4 weeks on, 2 weeks off. The primary endpoint was overall survival (OS), determined from time of randomization until death due to any cause. Adverse events (AE) of interest were coded according to MedDRA version 16.0. Results: At baseline, patient characteristics were balanced between groups within salvage designations. The rate of complete remission, with or without full hematologic recovery (CR/CRi/CRh) in both the S1 and S2+ groups was higher in the blinatumomab arm compared with the SOC arm (Table 1). Patients randomized to blinatumomab had a median (95% CI) of 11.1 (8.2, NR) months vs 5.1 (3.2, 7.1) months overall survival for S1 vs S2+ subgroup, compared with 5.5 (3.7, 9.0) months vs 3.0 (2.1, 4.0) months in the SOC arm (Figure 1). For both S1 and S2+ subgroups, blinatumomab patients had longer median survival time. Grade 3 or worse AEs were experienced by 61% and 83% of S1 patients in the blinatumomab and SOC group, respectively. These percentages were 68% and 75%, respectively, in S2+ patients. Grade 4 or worse AEs occurred in 34% and 51% S1 patients, and in 36% and 54% S2+ patients. Neurologic events of grade 2 occurred in 9% and 9% of S1 patients, and in 10% and 9% S2+ patients, respectively. Grade ≥3 cytokine release syndrome (CRS) was observed in 4% S1 and 5% S2+ patients receiving blinatumomab, and in no SOC patients.

Table 1.

| No prior salvage | | Any prior salvage | |
|------------------|------------------|------------------|
| Blinatumomab (n=271) | SOC (n=134) | Blinatumomab (n=271) | SOC (n=134) |
| CR/CRi (95% CI) | 48.8 (42.6, 55.0) | 14.3 (10.4, 18.2) | 49.7 (43.5, 55.9) | 14.5 (10.6, 18.3) |
| CR/CRi (95% CI) | 49.7 (43.5, 55.9) | 14.5 (10.6, 18.3) | 49.7 (43.5, 55.9) | 14.5 (10.6, 18.3) |

Summary/Conclusions: Patients in this trial receiving blinatumomab for R/R ALL achieved improved OS and remission rates compared with SOC regardless of prior salvage therapy. Improved OS compared with SOC in S1 patients supports earlier use of blinatumomab.

S479

DURABLE LONG-TERM SURVIVAL OF ADULT PATIENTS WITH B-ALL AFTER CD19 CAR (19-28Z) T CELL THERAPY

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Background: CD19-specific chimeric antigen receptor (CAR) T cells have demonstrated high initial responses in patients with relapsed B-ALL. However, clinical characteristics associated with the durability of response remain undefined.

Aims: We performed a retrospective analysis of our phase I clinical trial of 19-28z CAR T cells in adult patients with relapsed B-ALL (NCT01044069) with a focus to identify those patients who optimally benefit from 19-28z CAR T cell therapy with durable long-term survival and reduced toxicities.

Methods: Adults with relapsed B-ALL were infused with autologous T cells expressing the 19-28z CAR following conditioning chemotherapy. Disease burden was assessed by bone marrow biopsy immediately prior to T cell infusion; patients with <5% blasts were classified as minimal residual disease (MRD) cohort vs patients >5% blasts as morphologic disease cohort. Response assessment occurred at 4 weeks. Median follow-up duration was 18 months (range, 2-57.3).

Results: 51 adults received 19-28z CAR T cells; 20 in the MRD and 31 in the therapy with therapeutic cohort. Complete remission (CR) rates were comparable (95% and 77%, respectively). However, median event-free and overall survivals widely diverged among the 42 patients who achieved MRD-negative CR: not reached (NR) (95% confidence interval [CI]: 4.2-119.6) vs 6.3 months (95% CI, 4.8-8.9) (p=0.0005), and NR (95% CI, 15.3-NR) vs 17 months (95% CI, 8.5-36.2) (p=0.0189), in the MRD and morphologic cohorts, respectively. Subsequent allogeneic HSCT in either cohort did not improve survival (p=0.8). MRD cohort patients developed substantially less severe cytokine release syndrome (CRS) and neurotoxicity, and both toxicities significantly correlated with peak
CAR T cell expansion (p=0.0326 and p=0.0001, respectively). No case of cerebellar edema was observed.

Summary/Conclusions: Despite comparable initial CR rates regardless of pre-treatment disease burden, durability of 19-28x CAR T cell mediated remissions and survival in adult patients with relapsed B-ALL positively correlated to a low disease burden and do not appear to be enhanced by allogeneic transplant. Our findings strongly support the early incorporation of CD19 CAR therapy before morphologic relapse in B-ALL.

S480

STANDARD-RISK RANDOMIZATION OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA TRIAL AIEOP-BFM 2000 INDICATES EQUAL OUTCOME WITH REDUCED-INTENSITY DELAYED INSTITUTION IN ETV6-RUNX1-POSITIVE PATIENTS

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Background: ETv6-Runx1 fusion is a common genetic aberration in childhood acute lymphoblastic leukemia (ALL) and is associated with good prognosis in the context of contemporary treatment regimens. The required treatment intensity for this well-described biologic subgroup with low risk of relapse is not known so far. In trial AIEOP-BFM ALL 2000, feasibility of reduced delay of intensified treatment to reduce the burden of chemotherapy was tested in a randomized approach in the standard-risk group. Treatment reduction was not successful in the total cohort (8-year probability of disease-free survival (8y-PDFS, ± standard error) 89.2±1.3% for reduced intensified treatment, 92.3±1.2% for the standard treatment (log-rank P=0.04) due to evidence of more relapses observed in patients treated less intensively.

Aims: The retrospective subgroup analysis presented here focuses on the ETv6-RUNX1-positive patients included in the group of randomized standard-risk patients.

Methods: From 07/2000 to 06/2006, 4741 eligible patients with ALL (age range 1-17 years) were enrolled in the trial AIEOP-BFM ALL 2000 (NCT 00430138 (BFM) and NCT 00613457 (AIEOP)). Of those, 1164 patients were considered at standard risk of relapse, defined by lack of genetic high-risk criteria and absence of minimal residual disease at day 33 and week 12 of treatment (tested by immunoglobulin/T-cell receptor gene rearrangement polymerase chain reaction). They were randomly assigned to either receive the reduced-intensity protocol (P-II) or the standard intensified protocol (P-III) for delayed intensified treatment. P-II is shorter than P-III (duration 29 vs 49 days), the dose of dexamethasone in P-III is 30% lower, and the dose of vincristine, doxorubicin, and cyclophosphamide is 49 days), the dose of dexamethasone in P-III is 30% lower, and the dose of vincristine, doxorubicin, and cyclophosphamide are reduced by 50% as compared to P-II. The intention was to prove non-inferiority of the reduced-intensity treatment compared to standard treatment.

Results: ETv6-RUNX1-positive patients (n=367) accounted for 34% of randomized standard-risk patients (Age: <6 years n=260, ≥6 to <10 years n=79, ≥10 years n=28; early cytologic response evaluation in bone marrow on day 15 of induction treatment: M1 n=218, M2 n=74). Of those, 188 were treated with the experimental P-II, 179 received the standard P-II. With a median follow-up of 6.8 years, the as-treated analysis showed an 8y-PDFS of 94.5±1.7% for P-II (p=0.06) vs 92±1.8% for P-III (p=0.74). Cumulative incidence of relapse at 8 years was 3.3±1.3% and 4.3±1.6% (Gray P=0.09), and 8-year overall survival was 96.9±1.4% and 98.8±2.0% (P=0.27) for P-II and P-III, respectively. Analysis of ETv6-RUNX1-positive patients by age groups or treatment response on day 15 allowed no further refinement of prognostic subgroups.

Summary/Conclusions: There was no evidence of prognostic disadvantage in ETv6-RUNX1-positive standard-risk patients when treated with the reduced-intensity experimental arm. No clear age- or response-dependent differences could be revealed for this group, which is in line with the biologic understanding of this genetic subgroup. Hence, it might be postulated that treatment reduction might be feasible in this well-defined biologic subgroup. However, the presented data is not a sufficiently powered non-inferiority study question focused on the subgroup of ETv6-RUNX1-positive patients, but reflects a subgroup analysis with descriptive character. Therefore, any decision for treatment reduction should be considered carefully.

Biology and disease monitoring in CML

S481

A SECOND GENERATION LYOSOMOTROPIC AGENT DRIVES LEUKAEMIC STEM CELL DIFFERENTIATION AND SENSITIZES THEM TO TYROSINE KINASE INHIBITOR TREATMENT IN VITRO AND IN VIVO

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Background: Autophagy is a conserved catabolic process that delivers cytoplasmic constituents to the lysosomes. We have previously shown that the lysosomotropic agent hydroxychloroquine (HCQ) inhibits autophagy and sensitizes Chronic Myeloid Leukaemia (CML) stem cells (LSCs) to tyrosine kinase inhibitors (TKIs) treatment. However, the biological effects of autophagy inhibition in LSCs in vivo are currently unknown and remain to be investigated. Furthermore, recent clinical studies showed that maximum tolerated dose of HCQ does not achieve consistent autophagy inhibition in cancer patients. Therefore further pre-clinical studies using more potent 2nd generation lysosomotropic agents, alone and in combination with TKIs, are vital.

Aims: Here we aim to investigate the functional effects of autophagy inhibition in LSCs both in vitro and in vivo using the highly potent lysosomotropic agent Lys05. Additionally, we aim to address whether Lys05 achieves autophagy inhibition in the most primitive LSC populations in vivo and whether it targets LSCs more effectively than HCQ when combined with TKIs.

Methods: In this study, we used primary stem-cell enriched samples (CD34+ cells) derived from CML patients at diagnosis. For in vivo studies, we used a human patient-derived xenograft (PDx) model and an inducible transgenic CML model in which the expression of BCR-ABL is induced at a stem/progenitor level (Scl-Tau-BCR-ABL). To accurately measure autophagy flow in long term LSCs in vivo, we generated the transgenic mouse Scl-Tau-BCR-ABL/GFP-LC3 by crossing the Scl-Tau-BCR-ABL mouse with a mouse bearing the autophagy marker GFP-LC3 fused to GFP.

Results: Firstly, we show that Lys05 targets LSCs more potently than HCQ in vitro by achieving a 60% and a 35% reduction in number of CD34+CD38+ and CFSEmeanCD3+CD133+ cells respectively. Interestingly, Lys05 promoted a 40% loss of quiescent cells and induced myeloid differentiation of CD34+ cells. Functional long-term culture initiating cell (LT-CIC) assay demonstrated that, while HCQ had moderate effects, Lys05 decreased the number of LSC-derived colonies by 80%. Additionally, we show that Lys05 inhibits autophagy flow more efficiently than HCQ both in the Scl-Tau-BCR-ABL/GFP-LC3 model and in patient-derived progenitor cells. Analysis of bone marrow (BM) cells from Lys05-treated leukaemic mice (but not from HCQ-treated mice), showed a statistically significant 35% decrease (p=0.0469) in the most primitive population Lin-Sca+citasCD48+CD150+ followed by a 50% increase (p=0.0231) of progenitors Lin-Sca+citasCD48+CD150-. This result indicates differentiation of LSCs towards a more progenitor phenotype following potent autophagy inhibition. Finally, to test the in vivo effects of Lys05, we transplanted CD34+ cells into irradiated NSG mice. Remarkably, using this PDX model we show that while 3 weeks in vivo treatment with HCQ had no effects when combined with TKIs, Lys05 and TKI treatment nearly eliminated engrafted primitive Philadelphia positive CD34+CD38+ and CD34+CD133+ cells.

Summary/Conclusions: Overall, we demonstrate that lysosomal inhibition induces loss of quiescence and drives differentiation of LSCs in vitro and in vivo. Furthermore, our results show that Lys05 achieves autophagy inhibition in LSCs and effectively sensitizes LSCs to TKIs in vitro and in vivo. Therefore, 2nd generation lysosomotropic agents should be considered as a potential alternative to HCQ in order to eliminate LSCs and achieve cure for CML patients.

S482

FC GAMMA RECEPTOR 2B IS CRITICAL FOR BCR-ABL MEDIATED LEUKEMOGENESIS

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Background: Chronic myeloid leukemia (CML) is provoked by the chromoso-
mal translocation t(9;22) that gives rise to the oncogenic tyrosine kinase Bcr-Abl. Implementation of tyrosine kinase inhibitor (TKI) therapy resulted in significant clinical success but with TKIs failing to eradicate the disease initiating leukemic stem cell population (LSC), this treatment is not curative in the vast majority of patients. By using a transgenic CML mouse model, we previously showed that LSC persist despite complete Bcr-Abl kinase inhibition due to a lack of cell-intrinsic cell death mechanisms. Subsequently, we identified the ITIM carrying Fc gamma receptor IIb (FcγRIIb; CD32) to be 2.8-fold upregulated in Bcr-Abl+ versus control LSK (lin;ScA-1; c-kit+) cells using microarray and qRT-PCR.

**Aims:** In this study, we first aimed to validate Bcr-Abl mediated FcγRIIb upregulation on mRNA and protein level in leukemic cells. Next, we tested the effect of shRNA mediated FcγRIIb knock-down and depletion on CFC (colony forming unit) capacity, proliferation and leukemic signaling in vitro. Finally, we studied the disease-initiating potential of primitive CML stem and progenitor cells upon FcγRIIb knock down.

**Methods:** qRT-PCR and western blot analyses were applied using cell lines, primary bone marrow cells and HoxB8 immortalized murine bone marrow (BM) cells for studying FcγRIIb expression and signaling. In order to test the biology of CML cells in vitro, we performed CFU and proliferation assays. Moreover, we performed viral infection of S-FU treated SCLIT/Cbc-Abl BM using FcγRIIb:shRNA or scrambled control and subsequent transplantation, followed by analyses of the disease, including immune-morphology, phenotyping, and protein expression as well as histological analysis.

**Results:** Bcr-Abl increased FcγRIIb mRNA (13.2-fold, p≤0.001) and protein expression in primary murine lineage negative (lin-) BM cells. Reduction of FcγRIIb in immortalized SCLIT/Cbc-Abl progenitor cells significantly reduced CFU (colony forming unit) formation (p≤0.001) and it impaired the proliferation rate in these cells (2.27-fold, p≤0.001). Moreover, transplantation of SCLIT/Cbc-Abl shRNA:FcγRIIb BM cells (CD45.1+) into FVB/n wildtype (WT) CD45.2+ recipients reduced spleen weight (352 ± 59.13mg), as compared to scrambled shRNA (568.1 ± 101.72mg). FACs analysis revealed a decrease in GFP+;CD45.1+ BM cells (1.43-fold, p≤0.001) upon FcγRIIb knock down. Likewise, donor-derived Gr-1+ cells (Gr-1+;CD45.1+;GFP+) were reduced in the BM (1.28-fold, p≤0.001) of these mice. Flow-cytometric analysis of the stem cell compartment revealed decreased leukemic BM LSK cells (lin-; c-kit+, Sca-1+, CD45.1+, GFP-; 1.38-fold, p≤0.05) in mice transplanted with shRNA:FcγRIIb vs scrambled control. We noted similar effects upon FcγRIIb depletion of FcγRIIb+/+ vs FcγRIIb+/+ cells using western blotting, positively implicating FcγRIIb as a key mediator in CML biology.

**Summary/Conclusions:** FcγRIIb is upregulated in LSC derived from transgenic CML mice upon Bcr-Abl expression. Complete depletion or knock down of the receptor reduces CFU capacity and cell growth in CML cells and significantly impairs CML development and LSC burden in vivo, presumably due to impaired leukemic downstream signaling. Our data demonstrate that FcγRIIb is critical and disease specific making it a potential novel therapeutic target in CML stem cells.

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**S483**

**MYC-DEPENDENT REPRESSION MECHANISM OF THE MIR-150 TRANSCRIPTIONAL REGULATION IN CHRONIC MYELOID LEUKEMIA**

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**Background:** The expression of miRNAs is regulated at transcriptional and posttranscriptional levels. Dysregulation of miRNAs could directly induce or be a consequence of oncogenic pathways. Chronic myeloid leukemia (CML) is characterized by a 150-150 level translocation leading to an insufficient repression of its target, oncogene MYC. CML treatment with imatinib normalizes miR-150 levels. Thus miR-150 is crucial for CML biology, however, little is known about its upstream transcriptional regulation. MiR-150 is an inhibitor of oncogene MYC, which is required for BCR-ABL1-dependent leukemogenesis in CML blast crisis (CML-BC) patients. We previously demonstrated that the number of copies of BCR-ABL1 DNA is directly proportional to the number of CML cells. Measuring both DNA and RNA may enable us to understand the contribution of expression and cell number to the RNA-RQPCR response.

**Aims:** To compare BCR-ABL1 DNA Q-PCR and real-time Q-PCR monitoring of CML.

**Methods:** Fifty-nine newly diagnosed chronic phase CML patients from the ALLG CML study (TIDE II) trial were included in this sub-study. Samples were tested prior to commencing TKI treatment (baseline), at 1, 2, and 3 months, and every 3 months to 24 months (total 568 samples). Since we wanted to compare the reproducibility of the Q-PCR methods we selected patients who had achieved undetectable minimal residual disease (UMRD) by Q-RQPCR within 24 months, and an additional 40 patients unslelected for response. Q-RQPCR results were expressed on the International Scale (IS), whereas DNA results were expressed relative to the individual patient’s baseline. Quantification of BCR-ABL1 DNA using the Q-PCR method involves quantifying the number of copies of BCR-ABL1 DNA is directly proportional to the number of CML cells. Measuring both DNA and RNA may enable us to understand the contribution of expression and cell number to the RNA-RQPCR response.

**Results:** We first demonstrated that DNA dPCR and real-time Q-PCR gave comparable results: 45 samples from 6 patients were quantified by both methods with comparable results: 45 samples from 6 patients were quantified by both methods with comparable results: 45 samples from 6 patients were quantified by both methods giving 95% limits of agreement ranging from -1.19 to 0.88. Subsequently, DNA and mRNA values were compared in paired samples. The median BCR-ABL1 IS at baseline was 58% (range, 2.4% - 487%) versus 93% by DNA methods (range, 2.4% - 235%). Interestingly, BCR-ABL1 DNA was significantly higher than mRNA at 1, 2, and 3 months (Figure). There was good agreement between positive results from 6 months of TKI therapy onwards (mean bias -0.02; 95% limits of agreement from -1.15 to 1.11). Comparing the limit of detection, BCR-ABL1 DNA was detectable in 60/148 (41%) samples with undetectable mRNA.
Aims:
The first phase of the study was aimed to i) create a network of 4 labs in 3 European countries, with a total of 12 participating labs, to prospectively assess the feasibility of using deep sequencing for routine monitoring of BCR-ABL1 mutation status in CML patients. In the second phase, 159 consecutive patients were prospectively studied in parallel by Sanger Seq and by Deep Seq. Among the pts with low burden mutations detectable by Deep Seq, 34 had other known TKI-resistant mutations; 14 had only mutations with unknown clinical significance. Pts positive for mutations by Deep Seq were more frequent in the High and Resistant subgroup. All the pts who need to be switched to another TKI would benefit from sensitive BCR-ABL1 KD mutation detection by Deep Seq.

Methods:
In the first phase, Sanger Seq plus 8 T315I+ BaF3 cell line dilutions simulating mutation loads between 20% and 1% were distributed and analyzed in parallel by each of the 4 participating labs. In the second phase, 101 Failures and 25 Warnings (33%; Sanger Seq plus 8 T315I+ BaF3 cell line dilutions simulating mutation loads between 20% and 1%) were distributed and analyzed in parallel by each of the 4 participating labs. In the second phase, 159 consecutive CML pts were prospectively studied in parallel by Sanger Seq and by Deep Seq. Among the pts with low burden mutations detectable by Deep Seq, 34 had other known TKI-resistant mutations; 14 had only mutations with unknown clinical significance. Pts positive for mutations by Deep Seq were more frequent in the High and Resistant subgroup. All the pts who need to be switched to another TKI would benefit from sensitive BCR-ABL1 KD mutation detection by Deep Seq.
PATIENTS WITH IDIOPATHIC CYTOPENIA OF UNDETERMINED SIGNIFICANCE SHOW SIMILAR SURVIVAL PATTERNS AS LOW RISK MDS PATIENTS

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Background: Cytopenia is a hallmark in myelodysplastic syndrome (MDS), however, many patients with persistent cytopenia do not fulfill the criteria for MDS. These patients are now classified as idiopathic cytopenia of undetermined significance (ICUS) or if a mutation is detected as clonal cytopenia of undetermined significance (CCUS). Little is known about these new entities in regards to survival and prognostication.

Aims: In this study we want to compare ICUS patients with MDS patients having low- or very low-risk disease according to the IPSS-R. We also wanted to investigate if sequencing of the cohort could bring additional information in regards to overall survival.

Methods: All patients underwent a bone marrow biopsy, cytogenetics and a broad range of blood tests. Furthermore, all ICUS patients underwent a blinded morphology review by two experienced pathologists; these review data will be ready for presentation at EHA. ICUS was defined as persistent cytopenia for more than six months, no chromosomal aberrations and common causes of cytopenia were ruled out. The patients were sequenced with a targeted sequencing panel, either using a customized Haloplex panel or a customized sequencing panel for the Ion Torrent platform. We analyzed 20 genes which are the most commonly mutated genes in MDS.

Results: So far we included 157 patients, 122 were classified as ICUS and 35 as MDS and the median age is 65 and 68 years, respectively (p=0.27). We have sequenced 78% of the ICUS patients and 74% of the MDS patients. In total 53% and 73% of the ICUS and MDS patients had at least one mutation detected, respectively. If the patients carried a mutation, the median number of mutations was two in both the CCUS and the MDS group. The most commonly mutated genes were TET2, SRSF2, DNMT3A and ASXL1 in 38 patients (31%), n=16 (13%), n=10 (8%), n=10 (8%), respectively. There were no significant differences in the distribution between the two groups. Mutations in NRAS, KRAS, TP53 were only identified in one patient each. The overall survival between the ICUS and the low-risk MDS patients did not differ (p=0.18) (figure 1). We also subdivided the ICUS patients into non-clonal ICUS and CCUS, but observed no difference between these two groups (p=0.355).

Eight of the patients categorized as ICUS progressed to a myeloid neoplasm during the follow up, and of these seven had a detectable mutation at time of enrollment, only one ICUS patient without a detectable mutation progressed (p=0.08).

Summary/Conclusions: We here demonstrate that low-risk MDS and ICUS patients share similar survival patterns, however, larger studies with longer follow up are needed. Mutations are most commonly found in the epigenetic regulators in this cohort of ICUS and low-risk MDS, while mutations in classical tumor suppressors and oncogenes such as TP53 and NRAS are rare. Mutational screening seems promising in detecting patients at risk of progression, however, other biomarkers for prognostication are warranted.

AN UPDATE OF A PHASE II STUDY OF NIVOLUMAB (NIVO) OR IPILIMUMAB (IPI) WITH AZACITIDINE IN PTS WITH PREVIOUSLY TREATED OR UNTREATED MYELODYSPLASTIC SYNDROMES (MDS)

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Background: Outcomes of pts with MDS after hypomethylating agent (HMA) failure remain poor. Upregulation of PD-1, PD-L1 and CTLA-4 in MDS CD34+ cells after exposure and loss of response to HMA have been reported. Nivo and Ipi are monoclonal antibodies targeting PD-1 and CTLA-4, respectively, with clinical activity in solid tumors.

Aims: To evaluate the potential activity of immune checkpoint antibodies in patients with previously treated or untreated MDS.

Methods: We designed a phase II study of Nivo or Ipi in monotherapy or combination for pts with MDS. Pts with prior therapy with HMA were to be treated in one of 3 consecutive cohorts combining AZA add-back after 6 cycles of therapy if there was no response or progression. Pts with previously untreated MDS were to be treated in one of 3 consecutive cohorts combining AZA 75mg/m2 iv daily days 1-5 of a 28 day cycle with: cohort #1: Nivo 3mg/kg iv days 1 and 15 of a 28 day cycle; cohort #2: Ipi 3mg/kg iv on day 1 of a 21 day cycle; cohort #3: Nivo 3mg/kg iv on days 1 and 15 + Ipi 3mg/kg iv on day 1 of a 28 day cycle. The study design allowed for AZA add-back after 6 cycles of therapy if there was no response or progression. Pts with previously untreated MDS were to be treated in one of 3 consecutive cohorts combining AZA 75mg/m2 iv daily days 1-5 of a 28 day cycle with: cohort #4: Nivo 3mg/kg iv Days 6 and 20; cohort #5: Ipi 3mg/kg iv on day 6; and cohort #6. Nivo 3mg/kg iv on days 6 and 20 + Ipi 3mg/kg iv on day 6. The maximum size per cohort is 20 pts. The primary endpoint is to determine the safety of Nivo or Ipi as single agents or in combination with AZA. Secondary objectives included overall response rate (ORR) and assessment of biological activity. Responses were evaluated following the revised 2006 IWG criteria. The study included stopping rules for response and toxicity.

Results: A total of 63 pts have been enrolled, 54 (86%) are evaluable for response and toxicity including 21 treated with frontline AZA+Nivo, and 15 and 18 with Nivo or Ipi after HMA failure, respectively Median age is 69 years (range 39-85). The median number of treatment cycles was 3 (range 1-11). A total of 3 (27%) pts in the AZA+Nivo cohort, 6 (40%) in the Nivo cohort, and 3 (33%) in the Ipi cohort having related grade ≥3 non-hematologic AEs. Therefore, the stopping rule for toxicity was not met in any of the cohorts. Delays of therapy due to AEs were required in 9 pts due to: rash (N=1), adrenal insufficiency (N=1), coitalis (N=1), thyroiditis (N=2), pneumonitis (N=3), and nephritis (N=1). Early 8-week mortality occurred in 1 patient due to non-related intracranial hemorrhage. The ORR was 80% (13/21) in the AZA+Nivo cohort including 6 CR. The ORR was 0% and 30% (5/18) in the Nivo and Ipi arms, respectively. The stopping rule for response was met on the Nivo arm, and enrollment after patient 15 was stopped. Immunophenotypic analysis of stem cell and progenitor compartments was performed in 27 pts, including PD-1 and PD-L1 expression analysis in 16 pts. Increased PD-1 and PD-L1 expression on progenitor and stem cell compartments was observed in 3 and 4 pts, respectively. Treatment with PD-1 inhibitors could not overcome the aberrant differentiation patterns. No differences in response were observed based on PD-1 bone marrow expression.

Summary/Conclusions: Preliminary results indicate that PD-1 blockade with Nivo in combination with AZA in untreated higher-risk MDS pts is associated with a tolerable safety profile and clinical activity. Single-agent Ipi is capable of inducing responses in previously treated MDS pts. Single-agent Nivo did not show clinical activity.

ORAL RIGOSERTIB COMBINED WITH AZACITIDINE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) AND MYELODYSPLASTIC SYNDROMES (MDS): EFFECTS IN TREATMENT NAIVE AND RELAPSED/REFRACTORY PATIENTS

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Background: Azacitidine (AZA) is first line therapy for patients (pts) with higher risk MDS and demonstrated efficacy in older pts with AML (Dombret et al, Blood
Results: The combination of oral RIG and AZA was administered to 54 pts, of whom 40 had MDS; HMA-treatment-naive (N=23) and previously HMA-failed pts (N=17). 17 pts received prior HMA therapy: 12 AZA, 4 decitabine and 1 both. Ten pts had AML and 6 had CML. 2 MDS patients with 20–30% marrow blasts were also included in the AML analysis. Median age was 68 years; 67% of pts were male; and ECOG performance status was 0–1 in 95% of pts. Pts have received 1–37+ cycles of treatment (median, 3.5 cycles), with a median duration of treatment of 17 weeks (range 4 to 160+ weeks). Of the 10 pts with AML, 6 had relapsed AML, 2 secondary AML and 2 with AML transformation. Eight pts were evaluable for response. There were 3 responses seen, for an ORR of 37.5%, with responses in both secondary and refractory AML. Two additional pts had stable disease (25%). Responses were durable, with the longest response approaching one year (Table 1). Among 33 evaluable MDS pts, overall response by IWG criteria was 76%; complete remission (CR) in 8 (24%), concurrent marrow CR (mCR) and hematologic improvement (HI) in 10 (30%), mCR alone in 6 (18%), and HI alone in 1 (3%). Median duration of CR was 8 months for the combination. Median time to initial response was 2 cycles, and median time to best response was 3 cycles. The most frequently reported AEs were diarrhea (70%), nausea (50%), back pain (40%), constipation (40%), fatigue (40%), and peripheral edema (40%).

Table 1.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Gender</th>
<th>ECOG</th>
<th>Prior HMA</th>
<th>Response</th>
<th>Duration (m)</th>
<th>Median OS (m)</th>
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<td>17</td>
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<td>PR</td>
<td>6</td>
<td>18</td>
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<tr>
<td>Patient 3</td>
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<td>1</td>
<td>Yes</td>
<td>CR</td>
<td>15</td>
<td>24</td>
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</table>

Summary/Conclusions: The combination of oral RIG and standard-dose AZA was well tolerated in repetitive cycles in pts with AML and MDS. Response was observed both in HMA-treatment-naive pts (85%) and in pts failing HMA therapy (62%), suggesting the addition of RIG can overcome HMA clinical resistance by acting as a chromatin modifying agent. In AML, responses were seen in 37.5% of evaluable pts. Based on these results, continued study in AML is warranted. A Phase III study of the combination of oral RIG and AZA in pts with treatment naive MDS is planned.
Background: As key factors in gene post-transcriptional regulation, microRNAs (miRNAs) have been identified to play important roles in carcinogenesis in various tumors. Myelodysplastic syndrome (MDS) is a group of clonal myeloid disorders characterized by refractory quantitative and qualitative abnormalities of hemocytes and its pathogenesis is poorly understood. Some studies have shown that abnormal expressions of some miRNAs have close relationship with the pathogenesis of MDS. Recently, low RPS14 expression is found common in all kinds of myelodysplastic syndromes including patients without 5q deletion, but its mechanism remains unclear.

Aims: To determine the cause of RPS14 reduction in MDS except 5q-syndrome, influence of miRNAs on RPS14 expression was analyzed, and the role of specific miRNA on proliferation, differentiation and apoptosis of hematopoietic stem cells were evaluated.

Methods: Firstly, we predicted that miR-223 may target 3’UTR of RPS14 by bioinformatics software, then verified if the special miRNA could target RPS14 by assay of luciferase activity. Secondly, the miRNA expression level of miR223 were detected in the bone marrow BM selected from 28 MDS patients including ten RCUD patients, four RCDM patients, four RAEB-1 patients and four RAEB-2 patients, meanwhile, the miR223 expression status were tested in four kinds of cell lines including SKM-1, HL-60, K562 and THP-1 cell lines through qRT-PCR and RPS14 expression was detected by means of immunofluorescence (IF).

Thirdly, constructing lentivirus which carried miR223 overexpression vector and inhibitor were infected to the SKM-1 cell line and k562 cell line which had the highest level of RPS14, then apoptotic analysis was detected by flow cytometry method and proliferation was tested by CCK-8 assay. Fourthly, hemin (50 μM) was added to increase RPS14 expression and RPS14 expression was detected by means of immunofluorescence (IF).

Results: 1. We verified miR-223 could target RPS14 by assay of luciferase activity. 2. The miRNA expression level of miR-223 were detected in the bone marrow BM selected from 28 MDS patients including ten RCUD patients, four RCDM patients, four RAEB-1 patients and four RAEB-2 patients, meanwhile, the miR223 expression status were tested in four kinds of cell lines including SKM-1, HL-60, K562 and THP-1 cell lines through qRT-PCR and RPS14 expression was detected by means of immunofluorescence (IF).

2. We found that forced expression of miR-223 suppresses commitment of r-globin, CD235a and CD71 labeling, in contrast, underexpression of miR-223 promoted cell proliferation and inhibit cell apoptosis while infecting miR223 inhibitor lentivirus had the opposite effect in SKM-1 and K562 cell lines.

Summary/Conclusions: MDS patients had higher miR-223 expression compared with health controls. We demonstrated that miR223 could promote cell proliferation, inhibit cell apoptosis and suppress terminal erythropoiesis through target RPS14.
serial samples, we inferred the clonal relationships between original and relapsed samples in 21 patients (Fig B). Mutations from initial diagnosis reappeared in 17 patients. The relapse clone of 13 patients was identical to or clonally evolved from the initial AML clone (7 and 6 patients, respectively). Relapse clones of 4 patients evolved from an inherited clonal, distinct from the initial AML clone. The remaining 3 patients’ relapse clones appear to be clonally evolving in the initial AML clone. We assessed whether the mutation status at pre- and post-HCT has any impact on OS and relapse after HCT. With a follow-up duration of 6.9 years, patients with VAF ≥0.2% at day 21 in any gene showed worse OS (HR 2.9, p=0.006) as well as increased risk of relapse (HR 5.3, p=0.0003) (Fig C-D). Multivariate analyses verified that VAF ≥0.2% at day 21 was a transferred mutation from the donor through HCT, illustrating the value of longitudinal NGS-based monitoring strategies for AML patients after allogeneic HCT.

Background: There are no approved therapies for chronic GVHD (cGVHD) after failure of steroids. Both B and T cells play a role in the pathophysiology of cGVHD. In preclinical models, ibrutinib (ibr) reduced the severity of cGVHD through inhibition of Bruton’s tyrosine kinase (BTK) and interleukin-2-inducible T-cell kinase (ITK).

Aims: This phase 2 study evaluated the efficacy and safety of ibr in patients (pts) with steroid-refractory/refractory cGVHD in need of additional therapy. Methods: Eligible pts had ≤3 prior regimens for cGVHD and either >25% body surface area erythematous rash or a NIH mouth score >4. Informed consent was obtained from all pts. Pts were treated with ibr 420mg/d until cGVHD progression or unacceptable toxicity. The primary end point was cGVHD response based on 2005 NIH consensus response criteria. Secondary end points included rate of sustained response, change in Lee cGVHD symptom score, change in Lee cGVHD involvement including skin, mouth, and gastrointestinal system showed similar responses (~90%). Of 25 responders with ≥2 involved organs, 20 (80%) were sustained responders, 5 responders discontinued ibr and 4 were observed with a median follow-up of 61.6 months. Of 20 treated responders, 37 were stable, whereas 9 were cleared and 15 acquired (or selected) at relapse. Overall, serial samples and donor samples de-convoluted origins of relapse clone from all 20 patients. Among the 13 patients whose donor samples were sequenced, no mutation that was transferred from donor to recipient expanded at relapse. We then assessed whether the mutation status at pre- and post-HCT has any impact on OS and relapse after HCT. With a follow-up duration of 6.9 years, patients with VAF ≥0.2% at day 21 in any gene showed worse OS (HR 2.9, p=0.006) as well as increased risk of relapse (HR 5.3, p=0.0003) (Fig C-D). Multivariate analyses verified that VAF ≥0.2% at day 21 was a transferred mutation from the donor through HCT, illustrating the value of longitudinal NGS-based monitoring strategies for AML patients after allogeneic HCT.

Results: 186 | haematologica | 2017; 102(2)
ciliated to lower LFS. Donor CMV seropositivity was associated with lower GRFS and higher NRM and aGVHD. In high risk-AML, aGVHD and cGVHD were 36% vs 24% (p=0.03) and 39% vs 33% (p=0.80) for HAPLO and MSD pts, respectively. At two years, NRM and RI were 18% vs 10% (p=0.16) and 21% vs 36% (p=0.02) while LFS and OS were 61% vs 55% (p=0.14) and 67% vs 66% (p=0.28) in HAPLO and MSD pts; GRFS was 48% vs 40% (p=0.17). In multivariate analysis risk of grade IV aGVHD (HR: 2.20; 95% CI: 1.20-3.74; p<0.01) was increased after Haplo as compared to MSD and no difference was observed in LFS, OS, and GRFS, respectively. Conditioning regimen was associated with lower NRM and higher GRFS, while younger age and donor CMV status was associated with lower RI, higher LFS and OS. Results were confirmed in an analysis of the with the the propension score technique as for RI, NRM, LFS, OS and GRFS.

Summary/Conclusions: As per our registry based study in intermediate risk AML results of HSCT from matched sibling donor are superior to those of HAPLO-HSCT, while in high risk-AML relapse is lower in the HAPLO transplants and NRM, LFS and OS is similar.

S495 IMPACT OF POST-TRANSPLANT INFUSION OF DONOR T CELLS GENETICALLY MODIFIED WITH INDUCIBLE CASPASE 9 SUICIDE GENE (BPX-501 CELLS) ON CHILDREN WITH LEUKEMIA GIVEN ALPHA-BETA T-CELL DEPLETED HAPLO-HSCT

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Background: HLA-haploidentical allogeneic hematopoietic stem cell transplant (haplo-HSCT) offers an option for children with acute leukemia in need of a transplant and lacking an available HLA-identical donor. However, performing haploidentical-HSCT without any graft manipulation has historically been associated with a high risk of acute and chronic graft-versus-host disease (GVHD). T cell depletion reduces the risk of GVHD, but leads to delayed immune reconstitution, predisposing to serious infection and leukemia relapse due to the lack of a T-cell mediated graft-versus-leukemia (GvL). To address these challenges, we have infused mature BPX-501 T cells (donor peripheral lymphocytes which have been modified with the iCasp9 suicide gene) after αβ T-cell depleted haplo HSCT to facilitate immune reconstitution and GVH effect. BPX-501 T-cells are genetically modified with the iCasp9 suicide safety switch and a truncated CD19 marker. In the event of GVHD, the switch is activated by an infusion of the drug rimiducid (AP1903) resulting in rapid T cell apoptosis and GVHD reversal.

Aims: This study was performed to evaluate both safety and efficacy of BPX-501 T cell infusion post αβ T-cell depleted haplo HSCT in pediatric patients with high risk ALL and AML in CR1 and CR2.

Methods: A prospective Phase II-II study enrolling children with hematopoietic disorder who lack a matched donor. 38 patients have been enrolled and treated with αβ TCR depleted haplo HSCT after a myeloablative preparative regimen followed by BPX-T cell infusion to date; of them, 24 had ALL and 14 AML (21% CR1, 79% CR2). Median follow-up is 11 months (range 3-24).

Results: All patients engrafted and no secondary graft failure was recorded. Median time to neutrophil and platelet recovery was 16 days (range 8-33) and 11 days (range 7-19), respectively. With a median follow-up of 11 months (range 3-24 months), the cumulative incidence of NRM and relapse was 3.7% and 12.0%, respectively, while the disease-free survival probability was 84.2% (Fig 1). All aGVHD resolved (5 Grade I skin, 5 Grade II skin, 2 Grade III GI). One child received rimiducid to treat steroid-resistant grade II skin with complete resolution in 24 hours (Fig 2). There were 3 cases of chronic GVHD, 2 were mild; 1 severe and fatal in a patient whose donor had VZV reactivation during mobilization. CD3+ T cells reached 500 cells/μl by day 90, with normalized CD4/CD8 T cell ratio by day 180.

Summary/Conclusions: Engraftment was brisk and T cell recovery normalized by 6 months. Overall incidence of severe aGVHD was low and the safety switch was successfully activated with rimiducid infusion. Cumulative incidence of NRM compares favorably to historic controls at the lead center, where a value of of 2.4% for matched related donors (MR), 11.8% for matched unrelated donors (MUD) and 5% for αβ T cell depletion haplo HSCT (Haplo αβ) without any T-cell infusion was recorded (Bertaina, 2015 ASH). The cumulative incidence of relapse was 12.0% for BPX-501, 32.3% for MR, 22.2% for MUDs and 21.9% Haplo-αβ. Disease-free survival in the BPX-501 treated patients was 84.2% compared to 65.4% for MR, 66.1% for MUDs and 73.1% for Haplo-αβ. However, length of follow-up on the control cohorts differed from that of BPX-501 treated patients. These data suggest that BPX-501 T cells modified with the iCasp9 safety switch, infused after selective αβ T-cell depletion, are safe and result in a rapid immune reconstitution and a potentially stronger GV effect in children with high-risk leukemia who lack a matched donor.
Bone marrow failure and PNH

S496
HEREDITARY HEMATOLOGIC MALIGNANCIES: GENETIC COUNSELING IMPLEMENTATION IN A LARGE LEUKEMIA CENTER
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Background: Hematologic malignancies have rarely been targets for genetic evaluation, even in familial cases. Over the past decade, more than 12 genes have been identified to cause inherited predispositions to hematologic malignancies. Genetic counseling, testing, and surveillance protocols for these families are not well-established. Additionally, many families with high incidence of blood cancers do not have described syndromes suggesting additional genes remain to be identified.

Aims: To identify individuals with inherited susceptibilities to hematologic malignancies, the Hereditary Hematologic Malignancy Clinic (HHMC) was established in April 2014 at The University of Texas M. D. Anderson Cancer Center. The clinic provides clinical and research testing for patients with hematologic malignancies suspected to have inherited predisposition syndromes.

Methods: Individuals were referred to the HHMC for several indications: (1) bone marrow failure/aplastic anemia/hypocellular MDS, (2) personal history of hematologic malignancy with ≥1 first-degree relative or ≥2 second-degree relatives with hematologic malignancy, (3) personal history of multiple primary cancers, (4) germline evaluation of presumed somatic mutations identified on next-generation leukemia prognostic panels, (5) management and/or surveillance of a previously-identified genetic syndrome, or (6) solid tumor heredity.

Results: Methods were evaluated in 97/152 individuals (64%). Research testing was performed in 46/152 (30%), particularly in patients negative for known susceptibility genes or without features suggestive of a clinical syndrome. Nine (6%) individuals did not undergo genetic testing. Clinical testing identified 23/97 (24%) individuals with a germline susceptibility to hematologic malignancy. Seven probands (7%) were identified to have RUNX1 mutations associated with familial platelet disorder with myeloid malignancy (FPD-AML). Six (6%) were identified to have the telomere disorder dyskeratosis congenita; only one of them met clinical diagnostic criteria with the "classic triad" of symptoms. Three (3%) patients were identified to have Li-Fraumeni syndrome due to constitutional TPS3 mutations. Two adults (2%) were diagnosed with Diamond-Blackfan anemia and both of these individuals developed adult-onset myelodysplastic syndrome after a long latency period and prior spontaneous remission of their childhood anemia. Two young adults (2%) with Fanconi anemia were diagnosed, and one patient each with DDX41 mutation and CBL (Noo- nan-like syndrome with JMML) were identified. Counseling, testing, and surveillance of identified mutation carriers in many affected families is ongoing.

Summary/Conclusions: Individuals with hereditary susceptibilities to hematologic malignancies are not as rare as previously thought. Clinical evaluation of these patients through genetic counseling and testing is high yield for identified at-risk families. Research-based sequencing for novel mutations is indicated and ongoing.

S497
SECONDARY LEUKEMIAS IN GENETIC SUBTYPES OF CONGENITAL NEUTROPENIA (ELANE, HAX1, WASP, G6PC3, ETC.): A LONG-TERM FOLLOW-UP
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Background: Leukemia predisposition is well known in congenital neutropenia (CN) subtypes. By taking all patients with known and unclassified CN together the incidence of secondary leukemia accounts for more than 10 percent. Advanced molecular diagnostics and the identification of inherited and acquired gene mutations have improved our understanding of leukemic transformation in CN patients.

Aims: In the European SCNIR 449 patients with congenital neutropenia and 91 patients with cyclic neutropenia (CyN) have been enrolled since 1994. These 449 patients were evaluated by causal follow-up. Outcomes include changes from baseline to last follow-up in LDH ratio, GPI-deficient granulocytes, red blood cell transfusions received, MAVE, and Functional Assessment of Chronic Illness Therapy (FAC-IT)-Fatigue score in patients with at least 6 months of follow-up.

Methods: Here we report the leukemia incidence of genetic subtypes analyzing all available long-term data from the European Branch of the Severe Chronic Neutropenia Registry (SCNIR). In addition, we analyzed 91 patients with CyN with or without ELANE mutations.

Results: Results from genetic testing were available for 314 of 449 CN patients, of whom 118 patients revealed ELANE, 48 HAX1, 71 SBDS, 28 G6PT, 9 G6PC3, 7 WAS, 5 TAZ1 mutations and 27 other rare gene mutations (e.g. p.14, CXCR4), 135 patients remain unclassified. In addition, 48 of 91 patients with CyN revealed ELANE mutations. Secondary myelodysplastic syndrome (MDS) or leukemia occurred in 49 of the 449 CN patients and in 1 of the 48 ELANE-CyN patients. Acquired CSF3R nonsense truncating mutations have been detected in the bone marrow cells of about 80% of CN patients who progress to MDS or acute myeloid leukemia (AML) and around 30-35% of non-leukemic CN patients, supporting the association between the acquisition of CSF3R mutations and leukemic transformation. These mutations have been shown to be acquired in hematopoietic cells only and therefore are not the primary cause of AML. The time of first detection of CSF3R mutations and time of malignant transformation is highly variable. Some patients progressed to MDS/AML within a few months. In others, CSF3R mutant clones persisted for many years without progression to leukemia. The distribution by genetic subtypes and the frequency of CSF3R mutations is shown in the table below.

Table 1.

<table>
<thead>
<tr>
<th>Gene Mutation</th>
<th>Patients</th>
<th>MDS/Leukemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>n (%)</td>
</tr>
<tr>
<td>Total CN</td>
<td>448</td>
<td>49 (11.0)</td>
</tr>
<tr>
<td>ELANE</td>
<td>118</td>
<td>17 (14.4)</td>
</tr>
<tr>
<td>HAX1</td>
<td>48</td>
<td>6 (12.5)</td>
</tr>
<tr>
<td>SBDS</td>
<td>71</td>
<td>6 (8.5)</td>
</tr>
<tr>
<td>SLCE1A4</td>
<td>28</td>
<td>1 (3.6)</td>
</tr>
<tr>
<td>WAS</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>JAG1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>mutations without leukemia</td>
<td>35</td>
<td>16 (45.7)</td>
</tr>
<tr>
<td>unclassified</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total CyN</td>
<td>91</td>
<td>10 (10.9)</td>
</tr>
<tr>
<td>ELANE CyN</td>
<td>48</td>
<td>4 (8.3)</td>
</tr>
<tr>
<td>unclassified</td>
<td>43</td>
<td></td>
</tr>
</tbody>
</table>

*Gene mutations without leukemia: G6PC3 n=9, TAZ1 n=5, p14 n=4, digenic mutations n=4, CDH1 n=4, CXCR4 n=3, germ-line extracellular CSF3R n=2, C16orf57 n=2, Pearson syndrome n=2, LYST n=1

All subgroups benefit from G-CSF treatment. However, patients requiring maintenance doses of G-CSF above 8μg/kg/day are at greater risk of leukemic transformation.

Summary/Conclusions: Conclusion: The incidence of secondary AML reflects the genetic heterogeneity of CN.

S498
EFFECT OF ECLIZUMAB IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) PATIENTS WITH OR WITHOUT HIGH DISEASE ACTIVITY: RESULTS FROM THE INTERNATIONAL PNH REGISTRY
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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, progressive, life-threatening disease caused by somatic phosphatidylinositol glycan class A (PIGA) gene mutation in bone marrow stem cells. The International PNH Registry (NCT01374380) is a prospective, multinational, observational study to record the natural history of PNH and collect data on long-term efficacy and safety of treatment with eculizumab (ecu), a humanized monoclonal antibody approved for treatment of PNH.

Aims: Evaluate the effect of ecu in patients with PNH with or without high disease activity (HDA/never ecu-treated; no-HDA/never ecu-treated; no-HDA/ecu-treated; no-HDA/never ecu-treated). HDA is defined as lactate dehydrogenase (LDH) ratio ≥1.5x upper limit of normal within 6 months of baseline and history of any of the following: fatigue, hemoglobinuria, abdominal pain, dyspnea, anemia (hemoglobin <100 g/L), major adverse vascular event (MAVE; including thromboembolism [TE]), dysphagia, or erectile dysfunction. Patients were assessed at baseline (date of enrollment in never ecu-treated patients; date of initiation of ecu in ecu-treated patients) and at last follow-up. Outcomes include changes from baseline to last follow-up in LDH ratio, GPI-deficient granulocytes, red blood cell transfusions received, MAVE, and Functional Assessment of Chronic Illness Therapy (FAC-IT)-Fatigue score in patients with at least 6 months of follow-up.

Methods: Patients enrolled in the Registry as of December 5, 2016, were stratified by HDA and ecu treatment status into 4 groups: HDA/ecu-treated; HDA/never ecu-treated; no-HDA/ecu-treated; no-HDA/never ecu-treated. HDA is defined as lactate dehydrogenase (LDH) ratio ≥1.5x upper limit of normal within 6 months of baseline and history of any of the following: fatigue, hemoglobinuria, abdominal pain, dyspnea, anemia (hemoglobin <100 g/L), major adverse vascular event (MAVE; including thromboembolism [TE]), dysphagia, or erectile dysfunction. Patients were assessed at baseline (date of enrollment in never ecu-treated patients; date of initiation of ecu in ecu-treated patients) and at last follow-up. Outcomes include changes from baseline to last follow-up in LDH ratio, GPI-deficient granulocytes, red blood cell transfusions received, MAVE, and Functional Assessment of Chronic Illness Therapy (FAC-IT)-Fatigue score in patients with at least 6 months of follow-up.
Results: 4717 patients were enrolled; of these, 3670 had non-missing data on euc and HDA status, and were included in the current analysis (HDA/euc-treated, n=778; HDA/never euc-treated, n=636; no-HDA/euc-treated, n=111; no-HDA/never euc-treated, n=1138). Median (min, max) duration of follow-up after baseline was longer for the euc-treated patients compared with the never euc-treated patients for both the HDA and no-HDA groups (see Table). Results for changes from baseline to last follow-up in outcomes of interest are summarized in the Table. Data show that patients in the euc-treated cohort had higher burden of disease at baseline. Specifically, in the HDA population, a higher proportion of euc-treated patients had a history of MAVE (33.3% vs never euc-treated patients (13.7%)). A similar disparity at baseline was also observed in the no-HDA population (33.0% vs 11.0%, respectively).

Following euc treatment, the divergence in the proportion of patients with MAVE has substantially narrowed for the HDA patients (3.9% for euc-treated vs 3.3% for never euc-treated) despite longer follow-up for the treated patients. Similar findings were seen in no-HDA patients (5.3% vs 2.1% respectively). In patients with MAVE, treatment with euc was associated with meaningful improvements in mean (standard deviation [SD]) reduction from baseline in LDH ratio (-5.0 [3.7] vs -0.4 [2.3]) and proportion of red blood cell transfusion-free patients (37.6% vs 15.8%). The FACIT-Fatigue data, while limited, showed the HDA/euc-treated group experienced a greater mean (SD) score improvement than the HDA/never euc-treated group (4.1 [10.3] vs 0.5 [6.8] points).

Summary/Conclusions: Our analysis of real-world data from the International PNH Registry has demonstrated that treatment with eculizumab was associated with improved outcomes in patients with HDA. Our findings are consistent with the notion that patients with HDA, including those with a history of MAVE, should be treated with eculizumab.

S499

CONGENITAL AMEGAKARYOCYTIC THROMBOCYTOPENIA: FUNCTIONAL RESCUE OF A NOVEL MPL MUTANT IN PRIMARY HEMATOPOIETIC STEM CELLS USING CRISPR-CAS9

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Background: Thrombopoietin (Tpo) and its receptor, Mpl, are the principal regulators of early/late thrombopoiesis and hematopoietic stem cells maintenance. Mutations in MPL can drastically impact its function and be a contributing factor in multiple hematologic malignancies, including congenital amegakaryocytic thrombocytopenia (CAMT). CAMT is a rare inherited syndrome characterized by thrombocytopenia at birth, progressing to bone marrow failure and pancytopenia. The functional impact of CAMT mutations on Mpl is yet to be determined. Here we report unique familial cases of CAMT presenting with a previously unreported mutation: T814C (W272R) in the context of CAMT and HDA. Function of the deficient Mpl receptor was restored via GRASP55 over-expression (forcing ER-trapped Mpl to traffic to the cell surface). Genome editing performed on cells carrying the W272R mutation restored the WT sequence and the response to Tpo, with similar cell proliferation as WT Mpl cells. Finally, when applied to primary Mpl K39N/W272R CD34+ cells, CRISPR-based gene editing rescued surface expression of Mpl and rescue to Tpo, as assessed by flow cytometry. These data suggest that primary CD34+ cells were able to generate a similar number of megakaryocytic colonies as control CD34+ cells in a single colony assay. Non-edited cells failed to do so.

Summary/Conclusions: We report a new double in cis mutation of Mpl (K39N/W272R) in the context of CAMT. Function of the deficient Mpl receptor could be rescued using trans-complementation with GRASP55 over-expression and CRISPR-Cas9 genome engineering. Successful editing of primary hematopoietic stem cells indicates direct therapeutic applications for gene editing in this disease.

S500

DISCOVERY OF ORALLY BIOAVAILABLE SMALL MOLECULES FOR INHIBITION OF COMPLEMENT C5

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uraemic syndrome (aHUS) are well-characterized diseases of complement dysregulation. The only approved therapeutic for these diseases is Soliris® (Eculizumab, Alexion), a monoclonal antibody that binds and inhibits the cleavage of complement C5. Soliris® requires lifelong intravenous administration by a medical professional every two weeks. An orally bioavailable small molecule inhibitor of complement C5 to treat these and other complement-mediated diseases represents a potential paradigm shift in the treatment of diseases of complement dysregulation.

Aims: To demonstrate the utility of an orally available, small molecule Complement C5 inhibitor for the treatment of complement mediated disorders.

Methods: Surface Plasmon Resonance (SPR) and Fluorescent Polarization assays (FP) were used to evaluate the affinity and specificity of the binding interaction between complement C5 and small molecule inhibitors. Determination of binding site, mechanism of action and potency were achieved by X-ray crystallography studies, Wieslab ELISA, and a sheep erythrocyte hemolysis based assay. The ability of the small molecules to prevent the hemolysis of PNH erythrocytes was evaluated using a modified Ham test. Pharmacokinetic studies were performed in rodents.

Results: Here we describe a series of first in class, orally bioavailable small molecules that bind to C5 with high affinity and inhibit its cleavage into C5a and C5b. These molecules demonstrate desirable drug-like properties with molecular weights under 500 amu and IPSA<100 A2. A high-resolution co-crystal structure resolved a unique binding site on the 189 kDa C5 protein, and specific binding of these molecules to C5 has been demonstrated by surface plasmon resonance (SPR) and fluorescence polarization (FP) assays. The position of the binding site suggests that these molecules will inhibit C5 cleavage in patients with the R855H/C polymorphism, which confers resistance to eculizumab. The molecules inhibit the terminal complement complex activity with single digit nanomolar IC50 as measured by inhibition of hemolysis in a highly sensitive antibody-sensitized sheep erythrocytes assay. In addition, they inhibit MAC deposition on complement-activating surfaces and prevent the cleavage of C5 to C5a and C5b as confirmed by ELISAs that directly detect generation of C5a and C5b. This sets the stage for the complement-mediated hemolysis of PNH erythrocytes (Type III) in a dose-dependent manner. More broadly, this series of molecules has been profiled by in vitro and in vivo ADME disposition studies and exhibits oral bioavailability (%F=30-50) in pre-clinical species.

Summary/Conclusions: The results presented here highlight, for the first time, the discovery of an orally potential small molecule inhibitor of C5. The development of an orally available complement C5 inhibitor has the potential to provide a new therapeutic modality to treat both rare and common conditions where terminal complement cascade inhibition is desired.

haematologica | 2017; 102(s2) | 189
Quality of life, palliative care, ethics and health economics

SS01
QUALITY OF LIFE WITH MELPHALAN/PREDNISONE PLUS EITHER THALIDOMIDE (MPT-T) OR LENALIDOMIDE (MPR-R) IN NON-TRANS-PLANT ELIGIBLE NEWLY DIAGNOSED MULTIPLE MYELOMA: RESULTS OF THE HOVON87/NMSG18 STUDY


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Background: We recently reported the results of the phase III randomized HOVON87/NMSG18 study showing comparable efficacy of treatment with melphalan, prednisolone and thalidomide following by thalidomide maintenance (MPT-T) versus melphalan, prednisolone and lenalidomide followed by lenalidomide maintenance (MPR-R) (Zweegman S et al. Blood 2016;127(9):1109-1116). As not only efficacy but also potential toxicity affecting quality of life (QoL) guides the choice of treatment, health-related (HR) QoL is important.

Aims: To evaluate the HRQoL results of the HOVON87/NMSG18 study. Methods: Two validated HRQoL instruments (EORTC QLQ-C30 and MY20) were completed at baseline, after 3 and 9 induction cycles (3ID and 9ID) and after 6 and 12 months of maintenance therapy (6MT and 12MT). The subscales global QoL, physical functioning, pain, fatigue, constipation, diarrhea, nausea/vomiting, insomnia, disease symptoms, side effects of treatment and neuropathy were analysed. Change in HRQoL score over time between treatment arms was assessed by linear mixed models. Independent sample t-tests were used to determine changes from baseline. Minimal important difference (MID) within arms was defined as a difference in score of ≥1 standard error of measurement (SEM) or, if a subscale consisted of one parameter only, MID within arms was defined as a difference in score of ≥1 standard error of measurement (SEM) or, if a subscale consisted of one parameter only, MID of the arm was defined as a difference in score of ≥1 standard error of measurement (SEM). To determine clinically relevant superiority of one arm, a difference in score of ≥5 was used and in addition significance level was calculated.

Results: From 553 (90.2%) of the 613 patients who participated in the HRQoL part of the study a baseline questionnaire was available. Forty (15%) of patients randomized to MPT-T versus 88 (24%) of patients randomized to MPR-R completed therapy until 12 months of maintenance therapy. Change in HRQoL between arms over time: in MPT-T improvement of HRQoL over time as compared to MPR-R was found for the subscales diarrhea and insomnia. In contrast, MPR-R showed improvement over time for the subscales pain, constipation, fatigue, and neuropathy. As compared to MPT-T, difference in HRQoL per arm: In MPT-T MID was reached for the following subscales; global QoL increased after 9ID until 12MT (MID range 7-13), pain decreased at every time point (MID range -21 to -23), diabetes symptoms deceased after 9ID (MID -12), fatigue decreased during MT (MID 12) and insomnia decreased at each time point (MID range -14 to -26). In MPR-R the MID was reached for the following subscales; global QoL increased after 9ID until 12MT (MID range 8-14), physical functioning increased at 12MT (MID 13), pain decreased at every time point (MID range -14 to -26) and insomnia decreased at 6MT (MID -10).

Summary/Conclusions: Both treatment with MPT-T and MPR-R controlled pain and resulted in an improvement in global QoL, as compared to baseline after 9ID and during maintenance. Treatment with thalidomide initially resulted in less pain and disease symptoms. At all treatment stages thalidomide caused less diarrhea, fatigue and insomnia as compared to treatment with lenalidomide. In contrast, therapy with lenalidomide resulted in less side effects of treatment, less constipation and less neuropathy as compared to thalidomide at all stages of treatment. In addition, long term maintenance therapy with lenalidomide resulted in better global QoL, better physical functioning and less pain.

SS02
HEALTH-RELATED QUALITY OF LIFE RESULTS FROM THE PHASE III GALLIUM STUDY OF OBINUTUZUMAB-BASED AND RITUXIMAB-BASED THERAPY IN PATIENTS WITH PREVIOUSLY UNTREATED ADVANCED INDOLENT NON-HODGKIN LYMOPHMA


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Background: Maintenance of pretreatment health-related quality of life (HRQoL) and/or meaningful improvements in HRQoL are important for previously untreated indolent non-Hodgkin lymphoma (iNHL) patients (pts). GALLI-
UM (NCT01332968) is an open-label, randomized Phase III study of obinutuzumab (GA101; G) plus chemotherapy (chemo) followed by G maintenance (G-chemo) compared with rituximab (R) plus chemo followed by R maintenance (R-chemo) in pts with previously untreated INHL. In GALLIUM, G-chemo produced a clinically meaningful improvement in investigator-assessed progression-free survival (PFS) among follicular lymphoma (FL) pts (34% reduction in risk of a PFS event relative to R-chemo). Grade 3–5 and serious adverse events were more common with G-chemo.

Aims: To compare changes in HRQoL in FL pts receiving G-chemo and R-chemo during GALLIUM.

Figure 1.

Methods: Enrolled pts were aged ≥18 years with documented, previously untreated FL (grades 1-3a), advanced disease (stage III/IV or stage II with tumor diameter ≥7cm), ECOG performance status 0-2, and requiring treatment according to GELF criteria. Pts were randomized 1:1 to R or to G at 375mg/m² on day (D) 1 of each cycle (C) or G 1000mg on D1, 8, and 15 of C1 and D1 of C2-8, for 6 or 8 cycles depending on chemo (CHOP, CVB or bendamustine). Responders continued to receive R or G every 2 months (mo) for 2 years or until progression. The Functional Assessment of Cancer Treatment-Lymphoma (FACT-Lym) questionnaire (Webster et al. 2005) was used to assess overall HRQoL, physical and functional well-being, and disease- and treatment-related symptoms. FACT-Lym was administered on D1 of C1 and C3 during induction, at the end of induction, and at mo 2 and 12 during maintenance/follow-up. For each FACT-Lym scale, mean and 95% confidence interval (CI) were derived for recorded scores at each visit and changes from baseline. Minimally important differences (MIDs) were used to calculate the proportion of pts reporting improvement on the FACT-Lym lymphoma subscale (LYMS; ≥3 points), Trial Outcome Index (TOI; ≥6 points), and lymphoma total score (LYM-Total; ≥7 points). All pts gave informed consent.

Results: Of 1202 FL pts randomized (median age, 59 yrs; 53.2% female; median observation time, 34.5 mo [range 0-54.5]), 566/601 (92.5%; G-chemo) and 550/601 (91.5%; R-chemo) completed all FACT-Lym scales at baseline. Baseline demographics and disease characteristics were balanced between arms. At baseline, mean HRQoL scores were similar in the two treatment arms, with all pts having some impairment of physical function, functional wellbeing, emotional and social function. Over the course of treatment, mean HRQoL was similar in the two treatment arms. From end of induction onwards, pts in both treatment arms. From end of induction onwards, pts in both groups showed improvements in HRQoL across all scales compared to baseline, with 18.3% and 21.9% of pts, respectively, achieving a MID on the LYM-Total scale by mo 12. In both groups, the greatest improvements were observed in the physical subscale, with 27.3% of G-chemo and 24.4% of R-chemo pts achieving a MID.

Summary/Conclusions: In previously untreated FL pts in GALLIUM, G-chemo and R-chemo produced similar improvements in HRQoL. These results suggest that lymphoma-related symptoms were reduced by both treatments and that the resulting improvements in well-being were not abrogated by treatment-related side effects. When viewed in the context of longer PFS, these results meaningfully improve. There were no clear differences between arms in functional and social function. Over the course of treatment, mean HRQoL was similar in the two treatment arms. From end of induction onwards, pts in both groups showed improvements in HRQoL across all scales compared to baseline, with 18.3% and 21.9% of pts, respectively, achieving a MID on the LYM-Total scale by mo 12. In both groups, the greatest improvements were observed in the physical subscale, with 27.3% of G-chemo and 24.4% of R-chemo pts achieving a MID.

Background: Cancer patient support groups appear to provide an important source of support to many patients and carers. In recent years there has been an increasing focus in the UK for services to provide cancer support groups, however it is unclear what proportion of patients believe access to these support groups would improve their experience of living with and beyond cancer.

Aims: A patient experience survey was undertaken by the Haematology-Oncology GMCPB across 10 NHS hospital trusts, where there are a number of cancer support groups.

Methods: The sample for the survey included all adult (aged >16) patients with a confirmed diagnosis of a haematological cancer who attended a haematological oncology outpatient appointment during a 4 month period (June-September 2016). The survey was available for completion via paper form or online and was completed anonymously. A translation/interpretation facility was not provided for patients whose first language was not English (due to funding restraints).

Results: 277 responses were returned with 1 response excluded (non-haematological malignancy). Haematological diagnoses included acute leukaemia (n=40), chronic leukaemia (n=35), lymphoma (n=62), myeloma (n=102), MDS (n=15), MPD (n=12), other (n=2) and not specified (n=7). 257 (93.1%) patients had received anticancer therapy, 218 (78%) were receiving treatment at the time of survey and 154 (54%) were on active therapy. 197 (71.4%) patients had not wanting access to a support group, 23 (19%) wanted access, 51 (8.3%) were not aware of the possibility and 6 (1.8%) did not respond. 51.8% of patients were aware of the existing support groups, 38.8% were not sure, 29.8% were not aware and 18% did not respond. The cohort of patients who did or did not want access to a support group was another 88%. 88% of patients had been given a key worker (e.g. oncology nurse specialist, research nurse, advanced nurse practitioner or nurse clinician); of those the 88% were satisfied and 1% were partly satisfied with the support they had received with 11% not responding. 93% (n=231) of patients were satisfied with the information they had received at diagnosis and 90% (n=224) felt their diagnosis had been given sensitively. Only 20% of patients currently on treatment wanted access to a support group and 24% not on treatment wanted access to a support group. Date of diagnosis was divided into three groups. Grp A: before 2005 (n=15), Grp B: after 2005 (n=229) and not stated (n=14). There was no difference in the three groups when asked if they wanted access to a support group (13%, 22%, 7% respectively; p=0.3) or awareness that support group was available (40%, 57%, 50% respectively; p=0.6). There were additional comments from patients that support from family and online forums in addition to key workers was extremely valuable to them. On univariate analysis patients who were satisfied with their key worker support did not want access to a support group (p=0.04). There was no trend on wanting access to a support group and diagnosis (p=0.67), treating hospital (p=0.5), information given (p=0.6), need for in-patient treatment (p=0.3), quality of care (p=0.8) or satisfaction with overall care (p=0.8).

Summary/Conclusions: Our results suggest that a large majority of patients with hematological malignancy do not want access to a cancer support group but providing satisfactory support through key workers and other health care professionals is likely to achieve better patient experiences.

Acknowledgements: We would like to acknowledge the members of the GMCPB and patients for their contribution to the survey.

S503

EFFECTIVE KEY WORKERS REDUCE THE NEED FOR CANCER SUPPORT GROUPS: RESULTS OF A POPULATION BASED SURVEY FROM GREATER MANCHESTER CANCER PATHWAY BOARD (GMCPB)

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Methods: From April 2015 to February 2017, 152 consecutive patients with acute leukemia planned for remission induction chemotherapy were randomly assigned (1:1) to PICC (Arm A) or traditional CVC (Arm B) (Table 1). Inclusion criteria were age >18 years, expected survival >4 weeks, and need of central venous access (long-term >4 weeks). Exclusion criteria were ongoing uncontrolled systemic infection, presence of significant thrombosis/stenosis in arm or central veins, and inability to communicate and/or to sign informed consent. All insertions were followed by ultrasonography assessments and chest X-ray.

Results: 152 patients (130 AML and 22 ALL) with a median age of 47 years (range, 13-82), were randomized in the two arms. In the Arm A, 76 PICCs (power injectable PICCs, in new generation polyurethane, open-ended) were inserted in 76 patients. Single lumen PICCs (Fr) were inserted in 5 patients, and triple lumen PICC (Fr) was inserted in 1 patient. 68 PICCs were inserted in the right basilica vein, 5 PICCs were inserted in the left basilica vein and 3 PICCs were inserted in the left brachial vein. In Arm B, 76 traditional CVCs (ventured heparin-coated Vitalon CVC, Becton-Dickinson) were inserted by the Seldinger technique in other 76 patients. 45 CVCs were inserted in subclavian vein and 31 CVCs were inserted in internal jugular vein. Overall, the median duration of in situ catheter placement was 5 months: 6 months (range, 3-12) in the arm A vs. 3 months (range, 1-10) in the arm B. In the arm A, catheter-related thrombosis occurred in 8 patients (6 basilica veins, 2 brachial veins) and catheter-related bloodstream infections in 4 patients (4 coagulase-negative *Staphylococcus*; of them, 2 meticillin-resistant). In the arm B, 20 cases of catheter-related thrombosis (7 subclavian veins, 13 internal jugular veins) and 15 cases of catheter-related bloodstream infections (10 enterobacteriaceae; 5 coagulase-negative *Staphylococcus*; and, of them, 3 meticillin-resistant) were observed. Thus, PICCs were significantly associated with fewer major complications than traditional CVCs (catheter-related thrombosis: 10.5% in the arm A vs. 26% in the arm B, *p*=0.01 by χ² test; catheter-related bloodstream infections: 5% in the arm A vs. 19% in the arm B, *p*=0.007 by χ² test) (Figure 1). Questionnaire covering activities of daily living confirmed improvement of quality of life.

Summary/Conclusions: The preliminary observations of this ongoing Phase IV randomized study, focusing on front-line use of central venous access device in a high risk hematological population, suggest that the use of PICC represents an advance in terms of decrease of complication rate and improvement of quality of life for patients with acute leukemia.

THE SIMM STUDY: SURVEY OF INTEGRATIVE MEDICINE IN MYELOPROLIFERATIVE NEOPLASMS

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Background: Pharmacologic therapy stabilizes hematologic counts and splenomegaly in myeloproliferative neoplasms (MPN), however only partial symptom improvement typically occurs. Evidenced-based integrative care may address this need, however data is limited in patients with MPNs.

Aims: To investigate the association with symptom burden, quality of life, depression, and fatigue in MPN patients.

Methods: Patients were recruited via social media. Informed consent and online self-report surveys (Qualtrics) were completed capturing patient demographics, disease specific data, supportive care utilization, MPN symptom burden (MPN-SAF TSS), depression (PHQ-9 total >3 category), fatigue (BFI Usual), and an overall quality of life (QOL) single question assessment. ANOVA, chi square tests, and Wilcoxon rank sum tests methods were applied.

Results: Patients: A total of 1087 patient surveys were consented. Of these, 858 had 10 or more responses. There were 338 essential thrombocytosis (ET), 188 myelofibrosis (MF), 315 polycythemia vera (PV), and 17 other. In MF, DIPSS risk categories included low (8%), Int-1 (19%), Int-2 (29%), high (12%), and unknown (32%). Symptom association: Overall, patients had lower MPN related symptoms when participating in aerobic activity (*p*<0.001), massage (*p*=0.001), yoga (*p*=0.02), strength training (*p*=0.001), breathing exercises (*p*=0.001), and support groups (0.001). Overall quality of life was higher with aerobic activity (*p*<0.001), massage (*p*=0.02), yoga (*p*=0.02), strength training (*p*=0.001), breathing exercises (*p*=0.001), and support groups (*p*<0.001). Depression (PHQ-9 total >3 category) was lower in aerobic activity group (*p*=0.001), yoga (*p*=0.001), strength training (*p*=0.001), and meditation (*p*=0.2). Fatigue was lower in aerobic activity (*p*<0.001), massage (*p*=0.04), strength training (*p*=0.001), breathing exercises (*p*<0.001), and support groups (*p*<0.001). In subgroup analysis, ET and PV patients had lower symptom burden (MPN-SAF TSS) with aerobic activity (*p*<0.001, *p*<0.001), massage (*p*<0.01, *p*<0.02), and strength training (*p*=0.03,0.02). Support groups were found to be associated with lower symptoms in ET patients (*p*<0.03). In MF, breathing exercises (*p*<0.001) and support groups (*p*=0.03) were associated with lower symptom burden. See Table #1.

![Figure 1](image-url)

Summary/Conclusions: Integrative therapies are associated with improved symptom burden, quality of life, depression, and fatigue in MPN patients. Interestingly, unique patterns were associated within MPN subtypes. Further studies are needed to understand the benefits of integrative therapies in MPN patients.
P506
T CELL EXHAUSTION CHARACTERIZED BY COMPROMISED MHC CLASS I AND II RESTRICTED CYTOTOXIC ACTIVITY ASSOCIATES WITH ACUTE B LYMPHOBLASTIC LEUKEMIA RELAPSE AFTER ALLO-HSCT
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Background: B cell acute lymphoblastic leukemia (B-ALL) relapse contributes to the predominant mortality after allogeneic hematopoietic stem cell transplantation (allo-HSCT). However, the mechanism of B-ALL relapse after allo-HSCT remains unknown. Eradication of leukemia in allo-HSCT settings largely relies on graft-versus-leukemia (GVL) effects mediated by donor T cells. T cell exhaustion characterized by increased expression of inhibitory receptors including PD-1 and Tim-3 and impaired function may blunt the GVL effects and was reported in acute myeloid leukemia relapse after allo-HSCT, whether T cell exhaustion is involved in B-ALL relapse after allo-HSCT remains unknown.

Aims: To evaluate whether T cell exhaustion is involved in B-ALL relapse after allo-HSCT, and investigate the correlation of inhibitory ligands on leukemic cells, leukemic load and T cell exhaustion, as well as the impact of treatment outcome on T cell exhaustion.

Methods: Our study enrolled 18 B-ALL patients who underwent first hematologic relapse after allo-HSCT and 18 matched B-ALL patients in remission (without minimal residual disease MRD) and 14 healthy donors from April 2016 to November 2016 at the Peking University People’s Hospital. In all patients, transplant protocol and post-transplant time were matched in relapsed and non-relapsed patients. Post-transplant time were matched as follows: ≥14 days within 12 months ±1 months from 12 to 18 months, ±3 months from 18 to 36 months, ±12 months over 3 years. Extra-medullary relapse were excluded in our study. All patients had achieved full donor chimerism before relapse or bone marrow collection. Peripheral blood (PB) were collected at the same day of bone marrow collection in relapsed patients. For patients who received induction therapy, we prospectively collected BM at least once after therapy. Sample collection was performed after patients was informed consent and approval by the institutional Human Ethics Review Committee of Peking University People’s Hospital in accordance with the Declaration of Helsinki. Phenotypic and functional studies of T cells in those patients were performed using multi-color flow cytometry.

Results: In the current study, we observed that increased co-expression of PD-1 and Tim-3 was observed in both CD4+ and CD8+ T cells in relapsed settings. Moreover, both CD4+ and CD8+ T cells exhibited compromised proliferative capacity, cytokine production and cytotoxic potentials such as degranulation and granzyme B production (preferentially on CD4+ T cells) in relapsed patients. In addition, T cells in the tumor site are more easily exhausted than those in peripheral blood. Reversal of T cell exhaustion was associated with effective anti-leukemic response in relapsed patients who underwent re-induction therapy.

Summary/Conclusions: In conclusion, our study suggested that T cells experienced exhaustion comprehensively functional impairments in B-ALL relapse settings after allo-HSCT and reversal of T cell exhaustion was associated with effective anti-leukemic responses. These results also provide a foundation for the development of novel effective leukemia therapeutics, such as anti-PD-1 or PD-1-L1 therapy, by targeting T cell exhaustion.

P507
RUXOLITINIB/NILOTINIB COTREATMENT BETTER INHIBITS LEUKEMIA-PROPAGATING CELLS IN PHILADELPHIA CHROMOSOME-POSITIVE ALL
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RUXOLITINIB/NILOTINIB COTREATMENT BETTER INHIBITS LEUKEMIA-
P508
PREDICTING ANTI-LEUKEMIA ACTIVITY OF THE B-2-SELECTIVE INHIBITOR ABT-199 IN BCP-ALL BY FUNCTIONAL ASSESSMENT OF APOPTOSIS SIGNALING
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Background: Despite the reduction in treatment-related mortality, B cell acute lymphoblastic leukemia (B-ALL) remains the major cause of treatment failure in patients with de novo Ph+ALL. Clinical stage II restricted cytotoxic activity associates with effective anti-LPCs effect occurred with the combination treatment was derived by the transplantation analysis of BCR/ABL and JAK2 activities, which represents a promising anti-LPCs therapeutic approach for patients with de novo Ph+ALL.

Aims: To identify the potential molecular basis of LPC-mediated relapse, RNA sequencing (RNA-seq) and real-time reverse transcription-PCR (qRT-PCR) were performed to analyze JAK2 and BCR/ABL gene expression profiles and associated gene expression in common leukemic cells and cells of other phenotypes from Ph+ALL patients. To investigate whether selective BCR-ABL/JAK2 dual inhibition therapy could more effectively eliminate LPCs in vitro and in humanized Ph+ALL mice.

Methods: RNA-seq and qRT-PCR were performed to analyze the gene expressions of sorted LPCs and cells of other phenotypes from patients with de novo Ph+ALL. In order to assess the effects of the selective BCR-ABL and/or JAK2 inhibition therapy by the treatment with single agents or a combination of ruxolitinib and imatinib or nilotinib on Ph+ALL LPCs, drug-induced apoptosis of LPCs was investigated in vitro, as well as in vivo using sublethally irradiated and anti-CD122-conditioned NOD/SCID xenograft mouse assay. Moreover, western blot analyses were performed on the BM cells harvested from the different groups of recipient mice.

Results: Using RNA-seq and qRT-PCR, we found that JAK2 was more highly expressed in the sorted LPCs than in the cells of other phenotypes in patients with de novo Ph+ALL in vitro study, cotreatment with nilotinib and ruxolitinib induced significantly higher levels of apoptosis in LPCs. In humanized Ph+ALL mice model, treatment with the nilotinib and ruxolitinib combination, compared with either ruxolitinib or TKIs alone, led to the most significant reduction in human Ph+ALL engraftment in the recipients. Further evidence that the most effective B-2-selective BCR-ABL/JAK2 dual inhibition therapy was associated with the combination treatment was derived by the engraftment analysis of BCR/ABL and JAK2 activities expressing cells using a qRT-PCR assay and HE and IHC with anti-hcD19 staining. Moreover, the combination of nilotinib and ruxolitinib more effectively reduced the LPCs capacity through a more effective suppression of phospho-CrKL, JAK2 and STAT5 activities at the molecular level.

Summary/Conclusions: JAK2 was more highly expressed in the sorted LPCs than in other cell phenotypes in patients with de novo Ph+ALL. Furthermore, selective BCR-ABL/JAK2 dual inhibition with nilotinib/ruxolitinib more effectively eliminated LPCs than either ruxolitinib or TKIs alone. Therefore, this pre-clinical study appears to provide scientific rationale for simultaneously targeting BCR-ABL and JAK2 activities, which represents a promising anti-LPCs therapeutic approach for patients with de novo Ph+ALL.

Aims: To assess the efficacy of ABT-199 in BCP-ALL, to functionally evaluate factors mediating ABT-199 susceptibility or resistance and to identify markers indicative of post-transplant leukemia activity.

Methods: The activity of ABT-199 was assessed by cell viability assays in BCP-ALL cell lines (N=6) and patient-derived xenograft (pdx) samples (N=27), analyzing half maximal effective concentrations (EC50). Expression of apoptotic regulators was detected by western blot analysis. MCL-1 deficient cell lines were generated by CRISPR/Cas9 gene editing. BH3 profiling was used to measure the mitochondrial dependence of leukemia cells on anti-apoptotic BCL-2 family proteins. In vivo treatment of ABT-199 was performed in a set of three distinct ALL rdxs.

Results: Different sensitivities of ABT-199 were observed in a series of BCP- ALL pdxs and cell lines with heterogeneous anti-leukemia activities upon drug exposure. The majority of BCP-ALL samples showed sensitivity to ABT-199-induced cell death in the nanomolar range (EC50 <1µM) with four out of six cell lines and 20 of 27 pdxs, while ABT-199 insensitivities with EC50s of more than 1µM were identified in 26% of pdx leukemias. ABT-199 induces apoptosis both in non-sub-nmB and non-B cell proliferation by targeting anti-apoptotic molecules; however, the sequestration of drug-released BIM by anti-apoptotic MCL-1 might lead to resistance. Therefore, we investigated protein expression of both regulators and found the ratio (BCL-2/MCL-1) to be cor-
related with ABT-199 sensitivity (k = 0.71, p = 0.008), highlighting the importance of functional assessment of the direct target molecule and additional resistance mediating molecules. In line, MCL-1 knockout in two ABT-199-resistant cell lines led to sensitization towards ABT-199, however, resulted in different effects of sensitization, emphasizing that ABT-199 resistance is determined by the interplay of several apoptosis regulators. Therefore, we characterized the functional dependence of pdx leukemias on anti-apoptotic BCL-2 family members by 4-hour BH3 profiling. Mitochondrial dependence on BCL-2 (mitochondrial priming by the BAD-peptide measuring BCL-2, BCL-XL and BCL-W, and subtracting the response to the HRK-peptide measuring BCL-XL) was found to be tightly correlated with ABT-199 sensitivity. In contrast, ABT-199-resistant samples were characterized by low BCL-2-dependence and addition to other BCL-2 family members, including BFL1 and MCL1. Finally, we evaluated prediction in vivo ABT-199 sensitivity in a pre-clinical ALL pdx mouse model by functional BH3 profiling. Strikingly, high mitochondrial BCL-2-dependency was clearly associated with prolonged leukemia-free survival upon ABT-199-therapy (two pdds, log rank p = 0.0035 and <0.0001), in contrast to another leukemia with low BCL-2-dependence and in vivo ABT-199 resistance (log rank p = 0.144).

**Summary/Conclusions:** SCP-ALL displays heterogeneous ABT-199 sensitivities characterized by the level of the target molecule but also other interacting regulators. Functionally, mitochondrial BCL-2-dependency assessed by the BH3 profiling assay is clearly associated with ABT-199 sensitivity. Importantly, in vivo anti-leukemia activity of ABT-199 therapy in individual pdx leukemias is predicted by mitochondrial BCL-2-dependency, emphasizing the utility of identification of patients and guidance of future clinical application by functional assessment of apoptosis signaling.

**P509**

**CD45RA+ MEMORY T CELLS EXPRESSING AN NKG2D-CAR TARGET PEDIATRIC ACUTE LEUKEMIA**

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**Background:** Lymphoid and myeloid acute leukemia are the most frequent type of cancer and the most frequent cause of cancer related death in children. Relapse and refractory disease are the main clinical problems that current therapies are still unable to solve. One of the main NK cell activating receptors is NKG2D ligand group 2D (NKG2D). NKG2D receptor recognizes human MICA, MICB and ULBP1-6 ligands. These NKG2D ligands (NKG2DL) are expressed in leukemia cells and constitute suitable targets for immunotherapy.

**Aims:** The aim of this study was to analyze the NKG2DL expression on pediatric acute leukemia cells and determine their susceptibility to an NKG2D CAR based immunotherapy.

**Methods:** The expression of NKG2DL was analyzed in Peripheral Blood Mononuclear Cells (PBMCs) from patients suffering from acute leukemia, as well as in leukemia cell lines, by flow cytometry (FCM) using specific monoclonal antibodies directed against MICA, MICAB, ULBP1-6, ULBP-3, and a Fluorescence activated Cell Sorter (FACS) to detect CD45RA+ NK cells. Healthy donors were labeled with CD45RA microbeads and depleted using AutoMACS device. The HL20i4r-MNDantiCD19bbz lentiviral vector was derived from the clinical vector CL20i4r-EF1a-hgcOPT27 but contained the extracellular domain of MICA/B, and was produced by transient transfection of HEK293T cells with the vector pCAGG-VSVG and pCAG4-RTR2. Cytogenetic studies and array Comparative Genomic Hybridization were performed to analyze the genetical status of lympho-transduced memory T cells. The in vitro cytotoxicity of CD45RA-NKG2DCAR T cells against leukemia cells, healthy PBMC and Mesenchymal Stem cells (MSC) was evaluated by performing conventional 4-hour europium-TDA release assays or by FCM using CFSE and 7AAD labeling of target cells.

**Results:** NKG2DL were heterogeneously expressed in leukemia primary cells and cell lines. For B cell ALL primary samples, we found expression of MICA/B, MICAB, MICB, ULBP1-6 and a fluorophore in refractory transduced cell lines. Lympho-transduction of NKG2D–4–1BB–CD3z increased NKG2D surface expression in CD45RA+ memory T cells, which became consistently more cytotoxic than untransduced cells against leukemia cells. Additionally, no chromosomal aberrations nor cytotoxic activity against healthy PBMC or Mesenchymal Stem cells was observed in NKG2DCAR expressing T cells.

**Summary/Conclusions:** Our results show NKG2D–CAR redirected CD45RA+ memory T cells target NKG2DL expressing leukemia cells in vitro and could be a promising and safe immunotherapeutic approach for pediatric acute leukemia patients.

**P510**

**A BILINEAL ACUTE LYMPHOBlastic LEUKEMIA ORIGINATING AT A COMMON LYMPHOID PROGENITOR**

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**Background:** Genetic mutations are crucial events during leukemogenesis and provide specific markers for backtracking the cellular origin of acute leukemias up to immature uni- or multi-potent progenitor cells in the hierarchy of the hematopoietic system.

**Aims:** To characterize the clonal architecture and cell of origin in a case of biphenotypic (B-ALL) and B-ALL

**Methods:** Bone marrow cells obtained at diagnosis were used for all studies. Immunophenotyping was done by flow cytometry. T- and B-cell leukemia purification was performed by immunomagnetics methods and DNA extracted afterward. TCR-gamma gene rearrangement was studied in T- and B-leukemic cells independently by PCR spectratyping. Somatic mutations in purified T- and B-leukemic cells were identified by deep-sequencing using a panel of 160 genes frequently mutated in cancer (Human comprehensive cancer panel, Qiagen). Mutations were validated by Sanger sequencing. Myeloid and erythroid clonogenic progenitors were isolated from methylcellulose cultures, DNA extracted, and assessed for the presence of the H3F3A p.K28N mutation by Sanger sequencing.

**Results:** The patient was a 10 years old boy. At diagnosis, the bone marrow was infiltrated by 60% leukemic cells, with 2 immunophenotypically different populations: a common B-ALL (54%) and a pro-T-ALL (6%). The patient showed extensive intrathoracic masses. The X-ray showed a thoracal mass. TCR-gamma rearrangement was detected in purified (>95% pure) T-ALL and B-ALL cells, suggesting a common origin for both leukemic subpopulations. The B-ALL cells presented a c.35G>A p.G12D mutation in the KRAS gene, absent in the T-ALL. The T-ALL cells presented a c.35G>A (p.G12D) mutation in the NRAS gene, absent in the B-ALL. A c.1126_1127insTAGA (p.P376fs*10) mutation in the WT1 gene was also detected only in the T-ALL. A c.84G>T (p.K28N) mutation in the H3F3A gene was detected in both the B-ALL and T-ALL subpopulations, confirming the involvement of a Common Lymphoid Progenitor in the process of leukemogenesis. The presence of the H3F3A p.K28N mutation in the myeloid compartment would point to a multipotent myeloid-lymphoid rather than a lymphoid-restricted progenitor as the cell origin of the leukemia. Therefore, we cultured myelocervoid-committed progenitor cells in clonogenic cultures and sequenced the H3F3A gene. None of the 122 myeloid or erythroid clonogenic progenitors (41 CFU-GM, 73 BFU-E and 8 CFU-GEMM) presented the p.K28N mutation in the H3F3A gene.

**Summary/Conclusions:** Our results indicate the involvement of a Common Lymphoid Progenitor as the cell of origin in this case of biphenal ALL as well as the crucial role of H3F3A and RAS family genes in the leukemogenesis process coupled with B and T differentiation.

**P511**

**CYSTEINE AND GLYCINE-RICH PROTEIN 2 (CSRP2) TRANSCRIPT LEVELS CORRELATED WITH LEUKEMIA RELAPSE AND LEUKEMIA-FREE SURVIVAL IN ADULT B-CELL ACUTE LYMPHOBlastic LEUKEMIA WITH NORMAL CYTOGENETICS**

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**Background:** Outcome of adults with B-cell acute lymphoblastic leukemia (ALL) remains poor and relapse is the major cause of treatment-failure. CSRP2 is a novel biomarker in B-cell ALL especially in those with normal cytogenetics and studying their clinical significance and biological function will be helpful for risk-stratification, treatment decision and targeted therapy. CSRP2 (cysteine and glycine rich protein 2) maps to chromosome subband 12q21.1. which is frequently abnormal in diverse cancers. Increased CSRP2 transcript levels are associated with de-differentiation in hematopoietic cell lines and CSRP2 is expected to be a new in vivo and in vitro bundling factor that critically promotes breast cancer cell invasion and metastasis. However, the clinical significance and biological function of CSRP2 in B-cell ALL remains unknown.
Aims: To identify novel biomarkers in B-cell ALL based on bioinformatics analyses; to examine the expression and clinical significance of CSRP2 in adults with B-ALL; to explore effects of CSRP2 on biological function of B-cell ALL.

Methods: We did bio-informatics analyses to identify mRNA transcripts aberrantly-expressed in B-cell ALL. RT-qPCR (real-time quantitative polymerase chain reaction) was used to examine CSRP2 transcript levels in bone marrow samples from 236 adults with B-cell ALL compared with samples from normal. A prognostic value was assessed in 168 subjects. CSRP2-knockdown and CSRP2-over-expression cell models were constructed to study the biological function of CSRP2 in B-cell ALL.

Results: We selected 9 candidate genes for validation 7 of which proved significantly-associated with B-cell ALL. CSRP2 was the most differentially-expressed gene in our validation studies. CSRP2 was over-expressed in 228 out of 236 adults (97%) with newly-diagnosed B-cell ALL. In subjects with normal cytogenetics: those with high CSRP2 transcript levels had a higher 5-year cumulative incidence of relapse (CIR) and worse relapse-free survival (RFS) compared to subjects with low transcript levels (56% [95% confidence interval 53-59%] vs 19% [18-20%]; P=0.011 and 41% [17-65%] vs 80% [66-96%]; P=0.007). In multivariate analyses a high CSRP2 transcript level was independently- associated with CIR (HR=5.32 [1.64-16.78]; P=0.005) and RFS (HR=5.56 [1.87-16.53]; P=0.002). Functional analyses indicated CSRP2 promoted cell proliferation, cell-cycle progression, in vitro colony formation and migration. Abnormal CSRP2 expression was associated with resistance to chemotherapy; sensitivity was restored by down-regulating CSRP2 expression. CSRP2 activated ERK1/2 signaling pathway, regulated cell-cycle related protein and activated CREB signaling pathway, whose activation was associated with poor prognosis in adults with B-cell ALL.

Summary/Conclusions: CSRP2 was widely-over-expressed in adults with B-cell ALL. Determination of CSRP2 transcript levels in subjects with normal cytogenetics might inform therapy-decisions. Consideration could be given to down-regulating CSRP2 expression as a way to reverse drug resistance.

P512

THERAPEUTIC TARGETING OF PRE-B CELL RECEPTOR SIGNALLING IN CHILDHOOD ACUTE LYMPHOBlastic LEUKEMIA

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Background: Acute lymphoblastic leukaemia (ALL) is the most common malignancy in children and adolescents and relapsed ALL remains one of the leading causes of cancer-related deaths in children. Components of the precursor-B cell receptor (Pre-BCR) signalling pathway are hijacked in ALL cells and this dependence may be therapeutically targeted. A number of tyrosine kinase inhibitors (TKIs) targeting effectors of this signalling pathway are showing great promise in the clinic and warrant preclinical evaluation in paediatric ALL. They include Dasatinib (BCR-ABL SRC inhibitor), Fostamatinib R406 (SYK inhibitor), Ibrutinib (BTK inhibitor) and CAL-101 (PI3Kδ-8 inhibitor).

Aims: To preclinically evaluate these candidate TKIs, as novel, targeted drugs for leukaemia treatment.

Methods: ALL cell lines (Reh, Nalm-6, PreB 697 and its glucocorticoid resistant cell line, R406) and primary bone marrow samples (PDX cells) were used in the study. Cell viability was assessed by Resazurin. Pre-BCR expression (µHc, VpreB and A5) and functionality using a Calcium flux assay were detected by Flow cytometry. Intracellular phospho-flow cytometry was used to detect constitutive phosphorylation and activation in response to anti-CD20 antibody, as well as drug pharmacodynamic measures (p-BTK, p-SYK, p-AKT, p-ERK, p-P-PLC-ϒ2, p-BLNK). Apoptosis and cell cycle were analysed by flow cytometry using Annexin V and Propidium iodide. RQ-PCR was used to measure expression of CSRP2, Zeste, GR expression and phosphorylation were detected by western blotting.

Results: ALL cell lines were modestly sensitive to Dasatinib (mean GI50 5.33 µM, range 2.45-12.5 µM) and R406 (mean GI50 4.32 µM, range 2.88-5.83 µM). However, cells were resistant to Ibrutinib (mean GI50 15.9 µM, range 11.47-18.3 µM) and CAL-101 (mean GI50 52.08 µM, range 25.75-77.83 µM). Cell cycle arrest and significant apoptosis was seen with R406 and Ibrutinib treatment, while Dasatinib and CAL-101 were cytostatic, causing G1 arrest with no substantial cell death. Pharmacodynamic assays confirmed inhibition of the relevant drug targets. PDX cells showed greater sensitivity than the cell lines to Dasatinib (4 out of 16 patient samples <5µM), R406 (7 out of 16 patient samples <5µM), Ibrutinib (3 out of 15 patient samples <5µM) and CAL-101 (3 out of 15 patient samples <2µM). Pre-BCR positive ALL cell lines and PDX cells were sensitive to R406 and Dasatinib, with a Ph+ PDX confirming sensitivity to the latter. Combining TKIs with the glucocorticoid (GC), Dexamethasone showed synergism in ALL cell lines and was particularly notable for Dasatinib and R406 in PreB cell receptor positive lines. Synergism was associated with significantly enhanced apoptosis, an increase in expression of the GR target gene, GILZ and for Dasatinib, enhanced expression of the pro-apoptotic, Bim. Control REH cells (GC receptor negative) showed no synergism.

Summary/Conclusions: Significant sensitivity of TKIs targeting Pre-BCR signalling have been identified at clinically achievable concentrations. Dasatinib and R406 sensitivity was associated with Pre-BCR positive ALL and combination with Dexamethasone showed significant synergism in GC resistant cell lines and PDX samples. TKIs were also effective in some Pre-BCR negative ALL cells, however, predictive biomarkers need to be established. Confirmation of these data in preclinical models in vivo may define new therapies for high risk ALLs.

P513

BMP-4 LEVELS IN CHILDHOOD B-ALL OF LOW-/INTERMEDIATE-RISK GROUPS IDENTIFY CHILDREN WITH POOR OUTCOME

P514

TARGETING LOCALIZATION OF THE IL-7 RECEPTOR WITHIN LIPID RAFTS AS A THERAPEUTIC STRATEGY FOR T-CELL ACUTE LYMPHOBlastic LEUKEMIA

haematologica | 2017; 102(s2) | 195
Methods:
Lipid rafts floating on the cell surface, and is delocalized by PyQ. Moreover, we have previously reported into lipid rafts thereby amplifying its downstream signaling pathway. The IL-7Rα is recruited and concentrated into lipid rafts on the cell surface of human T-ALL cells. We have also proved that localization of the IL-7Rα among lipid rafts plays a crucial role in human T-ALL blasts isolated from 10 patients suffering of T-ALL and maintained frozen in a biobank. 

Results: In this study, we have shown that PyQ delocalizes the IL-7Rα away from lipid rafts on the cell surface of human T-ALL cells. We have also proved that localization of the IL-7Rα among lipid rafts plays a crucial role in human T-ALL blasts. Its delocalization leads to IL-7 signaling pathway inactivation, upregulation of BAD and BIM genes involved in apoptosis and T-ALL cells apoptosis. We furthermore assessed effect of PyQ on 10 samples of primary T-ALL blasts. All of them were sensitive to IL-7-dependent cell survival and revealed a marked response to PyQ treatment (Mean IC₅₀=5.7 ng/mL). For this work, T-ALL cells were co-cultured with murine stromal MS5 cells and PyQ has affected mainly T-ALL cell growth. No effect was observed on the stromal feeder cells, suggesting that injection of PyQ in vivo would not impact the stromal microenvironment in bone marrow. Finally, we provided evidence that PyQ delayed T-ALL progression in vivo, after treatment of immunodeficient NOD/SCID/γc⁻/⁻ (NSG) mice. We also work on primary T-ALL blasts isolated from 10 patients suffering of T-ALL and maintained frozen in a biobank. 

Summary/Conclusions: The findings of this study highlight the importance of the IL-7Rα localization in maintenance of T-ALL cells and may lead to the design of a new generation of anti-cancer drugs able to modulate the protein positioning into lipid rafts.
Aims: To determine the impact of IM administration after HSCT on patient outcome and identify a small subset of patients unlikely to benefit, emphasizing the need for rigorous MRD monitoring. The identified MRD thresholds should be validated in an independent dataset. Their applicability in the setting of RIC transplant or 2nd/3rd G TKI remain to be determined.

Background: Front-line imatinib (IM) plus chemotherapy followed by allogeneic hematopoietic stem cell transplantation (HSCT) is standard therapy for patients (pts.) with Ph+ ALL. Relapse after HSCT remains a major cause of treatment failure, and pts. in whom BCR-ABL transcripts are detectable after HSCT are at particular risk. Prophylactic and preemptive TKIs have been shown to reduce the relapse rate, but the optimal strategy and timing remain to be determined.

Methods: In this prospective, multicentre trial by the GMALL study group, adult pts. (≥ 18 y) with Ph+ ALL in CR at HSCT were randomly assigned (1:1) to receive IM prophylactically after SCT or pre-emptively upon detection of MRD. Inclusion criteria included engraftment, sufficient hematopoietic and organ function, no evidence of infections, and a total dose of IM was 500-600 mg.
twice as likely to develop G3/4 CRS than pts with <50% BM blasts (n=29) (63% vs 24%). Earliest-onset fever correlated with severity of CRS. CRS grade correlated with serum IL-6 levels. CRS-associated coagulopathy with fibrinogen levels <1.0 g/L was observed in 10% of pts. Neuropsychiatric AEs occurred during or shortly after CRS resolution, were self-limiting, and were more likely in pts with severe CRS or history of CNS leukemia or other CNS diseases. No G4 non-hematologic AEs were observed. Other AEs of special interest within the first 8 wk included G3/4 neutropenia with high (>38.3°C) fever (61%) and infections (G3/4, 22%). Prolonged G3/4 neutropenia (not resolved >28 days) was uncommon (3%).

Summary/Conclusions: This pooled analysis of global experience with CTL019 across 25 sites and 11 countries found no new safety issues. CRS and neuropsychiatric events, which are class effects of CAR T-cell therapy, were dose dependent. CTL019 appears similarly safe in pts with Down syndrome or prior allogeneic SCT and across age groups. Prolonged follow-up will be required to determine the long-term safety of B-cell aplasia.

P519

PROGNOSTIC IMPLICATIONS OF PRETREATMENT CYTOGENETIC SUBGROUPS IN ADULTS WITH RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA TREATED WITH INOTUZUMAB OZOGAMICIN


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Background: In the phase 3 InO-VATE study of relapsed/refractory acute lymphoblastic leukemia (R/R ALL) patients, inotuzumab ozogamicin (InO) showed improved complete remission or complete remission with incomplete hematologic recovery (CR/CRi) rates versus standard care (SC; 80.7% vs 29.4%; P<0.001) (NCT01564784; Kantarjian NEJM 2016 [data cutoff date: Oct 2 2014]). Aims: To assess the impact of baseline karyotype on response and toxicities in R/R ALL patients receiving InO from the InO-VATE study.

Methods: Full study details have been previously published. At screening, karyotyping was performed locally; ≥20 metaphase count was recommended for cytogenetic analysis. Karyotypes were interpreted using the International System for Cytogenetic Nomenclature. CR/CRi and minimal residual disease (MRD) negativity rates (defined as <0.01% bone marrow blasts as assessed at central laboratory) were compared using a chi squared test and Fisher exact test. Survival estimates were compared using a log-rank test. Data as of March 8, 2016, are presented. Informed consent was obtained from all patients. All analyses presented were not adjusted for multiple testing.

Results: Of 326 patients randomized, 284 had cytogenetic data at screening (InO-treated patients: 164; SC: 140). Of 164 InO-treated patients, 81% had ≥20 metaphase ≥20 metaphases, 4.9% hyperdiploidy >50, 4.9% aberrations involving mixed lineage leukemia (MLL), 1.8% hypodiploidy/near-triploidy, 1.2% Del (9p), 16.5% other chromosomal abnormalities, and 12.2% missing. Of 164 InO-treated patients, CR/CRi rate was 73% (95% confidence interval [CI] 66–80; Table) and MRD negativity rate was 99% (95% CI, 91–97). With InO, CR/CRi and MRD negativity rates were similar between the various cytogenetic subgroups (P=0.86). Significantly higher complete remission rates with InO were observed in R/R ALL patients with ≥20 metaphases), complex, other, and missing cytogenetic subgroups (P≤0.015) and numerically higher in the other cytogenetic subgroups. With InO, more patients with diploid (≥20 metaphases) karyotype proceeded to stem cell transplant versus other cytogenetic subgroups. With InO, the duration of remission (DoR) was significantly different between cytogenetic subgroups (P<0.0001), with diploid (≥20 metaphases) and other subgroups having the longest median DoR numerically and MLL subgroup having the shortest median DoR numerically; no significant differences in DoR were seen between cytogenetic subgroups with SC (P=0.7853). Significant differences in PFS were seen between cytogenetic subgroups with InO (P=0.0063); no significant differences were seen between cytogenetic subgroups with SC (P=0.5427). With InO and SC arms, overall survival (OS) differences between cytogenetic subgroups were not significant (P=0.1629 and 0.3040, respectively); however, although not statistically significant based on 97.5% CI for hazard ratio (HR), OS was numerically longer (HR <1) with InO versus SC in diploid (≥20 metaphases), MLL, complex, other, and missing cytogenetic subgroups. Generally, adverse event profiles did not vary by cytogenetic subgroup.

Summary/Conclusions: In patients with diploid (≥20 metaphases), complex, other, and missing cytogenetic karyotypes, CR/CRi rates were significantly higher with InO versus SC (P=0.0063). With InO, MLL, complex, other, and missing cytogenetic subgroups, OS favored InO versus SC, though not statistically significant. Safety profiles generally were similar to the overall study population.
A PHASE II STUDY WITH A SEQUENTIAL CLOFARABINE-CYCLOPHOSPHAMIDE COMBINATION SCHEME AS SALVAGE THERAPY FOR REFRACTORY AND RELAPSED ACUTE LYMPHOCYTIC LEUKEMIA (R/R) IN ADULT PATIENTS

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Background: Blinatumomab, a bispecific T-cell engager antibody construct, has shown antileukemia activity and tolerability in patients with relapsed/refractory B-precursor acute lymphoblastic leukemia (ALL).

Methods: Eligible patients (aged ≥18 years) had ≥5% blasts in the bone marrow, and minimal residual disease (MRD, measured by polymerase chain reaction or flow cytometry) was found in patients with relapsed/refractory ALL. Safety and key efficacy outcomes were assessed in the first two cycles, and patients were followed for a minimum of 1 year to the end of follow-up.

Results: The phase II study included 127 patients, 47 (37%) of whom had relapsed ALL, and 60 (47%) had refractory disease. The overall response rate was 59.8% (25 of 42 patients), with 21% (9 of 42 patients) achieving complete remission. No new safety signals were found.

Summary/Conclusions: Blinatumomab showed antileukemia activity in pediatric and adolescent patients with high-risk relapsed/refractory B-precursor ALL, including t(17;19) and AEs were consistent with those previously reported for relapsed/refractory ALL.

BLINATUMOMAB USE IN PEDIATRIC AND ADOLESCENT PATIENTS WITH RELAPSED/REFRACTORY B-PRECURSOR ACUTE LYMPHOCYTIC LEUKEMIA FROM AN OPEN-LABEL, MULTICENTER, EXPANDED ACCESS STUDY

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Summary/Conclusions: Blinatumomab showed antileukemia activity in pediatric and adolescent patients with high-risk relapsed/refractory B-precursor ALL, including t(17;19) and AEs were consistent with those previously reported for relapsed/refractory ALL.

PRODUCT CHARACTERISTICS ASSOCIATED WITH IN VIVO EXPANSION OF ANTI-COD1 CAR T CELLS IN PATIENTS TREATED WITH AXICABATIGNE CILOUCELE (AXI-CELI)


Background: The incidence of acute lymphoblastic leukemia (ALL) is increasing, with nearly 6600 new diagnoses expected in 2016, of which >40% will
Methods: Eligible pts were ≥18 years of age with relapsed/refractory ALL (Ph+ pts eligible). ≥25% bone marrow lymphoblasts, adequate organ function, leukemia therapy and high disease burden (median, 81%, bone marrow lymphoblasts). No pt (0/3) experienced a DLT at the 2 × 10⁶ dose, and phase 1 was then expanded to 6 pts at the 2 × 10⁶ dose. One pt experienced a grade 5 adverse event of multi-organ failure due to cytokine release syndrome (CRS), and subsequent pts (n=4) received 1 × 10⁶ CAR T cells/kg. Across all pts, the most common grade ≥3 adverse events were cytokopenia (80%), febrile neutropenia (50%), pyrexia (40%), and transaminitis (40%). Grade ≥3 CRS and neurologic events were reported in 20% and 40% of pts, respectively. Cerebral edema was not observed. All CRS events resolved (except the grade 5 event); neurologic events resolved in 5 of 6 pts (1 grade 3 neurologic event ongoing at cutoff). Anti-CD19 CAR T cells achieved peak expansion within two weeks of infusion. Of the 8 efficacy evaluable pts, 6 (75%) achieved remission (including CR and CR with either partial or incomplete hematopoietic recovery) by day 28 disease assessment or earlier. All remissions (100%) were minimal residual disease-negative. Of the 6 pts achieving minimal residual disease-negative CR, two eventually relapsed, one with CD19- disease and one with CD19+ disease. Safety and efficacy data were similar across KTE-C19 doses. Updated pt number, follow-up, and biomarker data will be presented.

Summary/Conclusions: No DLTs were observed with KTE-C19 in adult pts with high BM disease burden; one pt with high disease burden had grade 5 CRS after completion of the DLT cohort. Manufacturing was successful in all pts; most pts achieved a minimal residual disease-negative CR. These results demonstrate promising efficacy with a manageable safety profile. Based on these results, ZUMA-3 continues to enroll pts, adding measures to further enhance safety and with planned expansion to phase 2.

P524

EXPOSURE-ADJUSTED ADVERSE EVENT COMPARING BLINATU- MOMB WITH STANDARD OF CARE CHEMOTHERAPY IN ADULTS WITH RELAPSED/REFRACTORY B-PRECURSOR ACUTE LYMPHOBlastic LEUKEMIA FROM A RANDOMIZED PHASE 3 STUDY

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Background: Blinatumomab, a bispécific T-cell engager antibody construct, has shown improved overall survival vs standard of care (SOC) chemotherapy in patients with Philadelphia chromosome−negative relapsed/refractory B-prec urser acute lymphoblastic leukemia (ALL) in a randomized phase 3 study (N Engl J Med 2017;367:836-847).

Aims: We compared the incidence of adverse events (AEs) observed with blinatumomab vs SOC after adjusting for varying treatment exposure times for a more comprehensive evaluation of safety and tolerability.

Methods: Adults (aged ≥18 years) with relapsed/refractory B-precursor ALL (refractory to primary induction therapy or salvage therapy, first relapse <1 year, second or later relapse, or relapse after allogeneic hematopoietic stem cell transplantation) were randomized to receive either blinatumomab or SOC (1 of 4 predefined regimens). Blinatumomab was dosed by continuous intravenous infusion (4 weeks on/2 weeks off) for up to five induction cycles (9 weeks on/2 weeks off) for up to five consolidation cycles (1-7 of cycle 1) or for up to five consolidation cycles (4 weeks on/8 weeks off) allowed for up to 12 months. Exposure-adjusted event rates were calculated as the number of events x 100/total exposure time (shown in the table).

Results: Median (range) number of cycles was 1 (1-4) for SOC and 2 (1-9) for blinatumomab. The highest exposure-adjusted event rates (per 100 patient-years) were for pyrexia (507 SOC vs 376 blinatumomab), anemia (987 vs 229), thrombocytopenia (750 vs 126), and neutropenia (351 vs 121), all of which were lower for blinatumomab than for SOC. Febrile neutropenia (365 vs 93) was also lower for blinatumomab than for SOC (p<0.0001). Exposure-adjusted event rates for neurologic events were 743 for SOC vs 472 for blinatumomab, with median time (range) to onset of 7 (1-43) days and 7 (1-190) days, respectively, and grade ≥3 cytokine release syn drome (CRS) rates were 0 for SOC vs 10 for blinatumomab. The most frequent grades of any AEs in both arms were cough (24% for SOC vs 12% for blinatumomab) and diarrhea (2% for SOC vs 1% for blinatumomab).
Table 1.

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**Summary/Conclusions:** In this study, blinatumomab showed an AE profile consistent with that previously reported for relapsed/refractory ALL, including similar rates of manageable CRS and neurologic events. Exposure-adjusted event rates were generally higher in SOC vs blinatumomab, including for cytopenias and infections.

**P525**

**FACTORS ASSOCIATED WITH STEM CELL TRANSPLANTATION OUTCOMES IN PATIENTS WITH RELapsed/REFRACTORY ACUTE LYMPHOBlastic LEUKEMIA TREATED WITH INOTUZUMAB OZOGAMICIN VERSUS CONVENTIONAL CHEMOTHERAPY**


**Background:** Inotuzumab ozogamicin (InO) therapy in relapsed/refractory acute lymphoblastic leukemia (R/R ALL) resulted in superior complete remission (CR)/CRi with incomplete hematologic recovery (CRi) rates versus (vs) conventional chemotherapy (C) in the Phase 3 INO-VATE trial (NCT01564784; Kantarjian NEJM 2016 [data as of October 2, 2014]. More InO v C patients (pts) proceeded to hematopoietic stem cell transplantation (HCT); 41% [45/109] v 11% [12/109]; P=0.001).

**Aims:** To assess factors associated with outcomes after allogeneic HCT in patients with R/R ALL who were previously treated with InO.

**Methods:** Full details have been published. Informed consent was obtained from all patients. Multivariate analyses (MVA) using Cox regression modeling were conducted to determine predictors of non-relapse mortality (NRM) and overall survival (OS).

**Results:** As of March 8, 2016, 108/326 pts underwent allogeneic HCT (InO n=77; C n=31). Baseline characteristics were generally similar, except baseline platelet values were lower in InO v C pts. More InO v C pts achieved minimal residual disease negativity during study therapy (MRDneg [best status]; 71% v 26%; P=0.0001). Less InO v C pts received additional therapy before HCT (14% v 55%, P<0.0001). NRM rates were higher in InO v C pts at 1 year (yr; 36% [95% CI 26–47] v 20% [8–36]) and 2 yrs (39% [27–51] v 31% [21–41]), but relapse rates were lower (1 yr, 23% [15–33] v 29% [13–48]; 2 yrs, 33% [22–44] v 46% [24–65]). No significant difference in post-HCT survival was detected in InO v C pts; however, visual inspection of the curve suggested the survival probability varied before and after 15 months post-HCT (1 yr, 44% [95% CI 33–55] v 65% [44–79]; 2 yr, 39% [28–50] v 34% [15–54]). Fatal veno-occlusive disease (VOD) was observed in 5 InO pts (all during the first 100 days from the date of HCT) and no C pts. MVA showed that conditioning regimens without dual alkylators and thiotaepa were associated (2-sided; P<0.05) with lower risk of NRM and post-HCT survival, respectively.

**Summary/Conclusions:** Compared with C, InO permitted more pts with R/R ALL to proceed to HCT in CR/CRI with MRDneg (best status). Despite increased NRM and fatal VOD, long-term survival was attainable in InO pts. In pts previously treated with InO, interventions to reduce NRM and improve OS after HCT include avoiding dual alkylator conditioning regimens, especially those containing thiotaepa.

**P526**

**DESIGNING THE NEXT GENERATION CD33-TARGETING ADC: IMGN779, SELECTED FOR POTENCY, NOVEL MECHANISM AND PRECLINICAL TOLERABILITY, WITH HIGH ACTIVITY IN DISSEMINATED AML MODELS AND IN MULTIDOSE REGIMENS**


**Background:** Antibody-drug conjugates (ADCs) targeting CD33 are promising therapeutic agents in AML, where challenges are achieving efficacy while maintaining tolerability. Here, we report the payload/ linker design and selection resulting in a high-Therapeutic Index (TI) ADC with favorable preclinical toxicology profile across multiple species and preclinical activity disseminated AML models and in multi-dose regimens. IMGN779, the final ADC design, is comprised of an indolino-benzodiazepine mono-imine DNA-alkylating payload, DGN462, coupled by a cleavable N-succinimidyl-4-(2-pyridyldithio)-2-sulfobutanoate (s-SPDB) linker to a CD33-targeting antibody.

**Aims:** Select the best ADC out of multiple preclinical anti-CD33 ADC candidates, and assess its activity in vitro and in vivo in AML models.

**Methods:** Unconjugated payloads were evaluated in vitro for cytotoxicity on human AML cell lines. Payloads were compared, as CD33-targeting conjugates, in vitro for cytotoxicity on human AML cell lines and in vivo for tolerability in mice and T1 against human AML xenografts. ADCs with favorable and non-cleavable linkers were evaluated for cytotoxicity on MDR-positive and -negative AML cell lines, for tolerability in mice and T1 in AML xenografts. IMGN779, the final ADC design, was evaluated in vivo for toxicity in rats and cynomolgous monkeys. IMGN779’s antitumor activity was evaluated in disseminated models and in fractionated- and multi-dose models in AML xenografts.

**Results:** First, we selected a high affinity antibody to CD33 with retained ADC activity. Next, given concerns for long-term toxicity of DNA crosslinkers, we prepared DNA alkylating (single strand DNA damage) and DNA crosslinking (double strand DNA damage) versions of our novel IgG payload class. Both versions had comparable IC50s on human AML cell lines as free drugs (12–260 vs. 5–77 µM) and as CD33-targeting ADCs (0.7 vs. 0.5 µM). However, in vivo, the CD33-targeting DNA alkylating ADC had a 5-fold higher MTD (maximally tolerated dose) in mice and 5-fold larger T1 in AML xenograft models (MTD 950 vs. 180 µg/kg, by payload, T1 of 95 vs. 19). In addition, the DNA crosslinking version led to delayed systemic toxicity at MTD, not seen in the DNA alkylating version even at its 5-fold higher MTD. Thus we selected the DNA alkylating version for further development. To determine the optimal linker design, we created ADCs with three different linkers, one non-cleavable and two cleavable, and based on improved in vitro efficacy (IC50) and in vivo safety/efficacy (MTD, T1), the s-SPDB cleavable linker with the DNA alkylating payload was chosen as the lead clinical compound, and named IMGN779. In multiple species, IMGN779 had a consistent toxicity profile (mouse, rats and monkeys), producing reversible cytopenias with no or minor changes in transaminases and without histologic evidence of hepatotoxicity. Importantly, IMGN779 was highly active at a single dose 10 µg/kg (payload) in an MV4-11 (FLT3-ITD+) disseminated AML xenograft model, producing an 90% increased life span, and was well-tolerated and highly active in repeat dosing regimens (10 or 30 µg/kg, qw 3 x 3) in a HL60 AML xenograft model. Similarly, in a MV4-11 xenograft model of single 1 µg/kg (payload) dosing (5 µg/kg, qw 2 x 2 or qw 3 x 3) the generated 33% more long-term tumor-free survivors compared to single-dose (10 µg/kg), demonstrating tolerability and enhanced efficacy in multi-dose and fractionated regimens.

**Summary/Conclusions:** IMGN779, designed as the next generation CD33-targeting ADC, utilizes a novel DNA alkylating DGN462 payload and a cleavable disulfide linker, selected to maximize anti-AML activity and preclinical safety. IMGN779 is highly active in multiple AML xenograft models, including models with poor prognostic factors, and is well-tolerated in preclinical repeat dosing regimes, where an additional benefit was achieved with a fractionating the dosing regimen over a single high dose. These results provide the foundation for the clinical evaluation of IMGN779 in AML.

**P527**

**THE MIXED LINEAGE LEUKEMIA FUSION PARTNER ENL RECRUITS TOLERABILITY, WITH HIGH ACTIVITY IN DISSEMINATED AML MODELS AND IN MULTIDOSE REGIMENS**

K. Hetzner, M. Garcia-Cuellar, C. Büttner, K. Slany

**Background:** In mixed lineage leukemia (MLL) fusion partner ENL is frequently found juxtaposed for the clinical evaluation of IMGN779 in AML.

**Aims:** Select the best ADC out of multiple preclinical anti-CD33 ADC candidates, and assess its activity in vitro and in vivo in AML models.
Aims: This work examines how ENL influences PRC1 repressive activity. Methods: The effect of ENL on transcriptional activity of model promoters and endogenous transcriptional control elements was studied by biochemical and molecular biology methods.

Results: Here we demonstrate that ENL overcomes polycomb induced silencing through recruitment of polymerase associated factor 1 (PAF1) a chromatin remodeling factor. This results in an ability to bind PAF1 conferring the ability of ENL to neutralize polycomb-mediated repression in an elongation reporter system and also during transformation of primary cells by MLL-ENL in vivo. Inactivation of polycomb by ENL was accompanied by ubiquitination of histone H2B, the hallmark activity of PAF1 alf elyzed enzymes. On a global scale, exposure to the transient RNA-Seq demonstrated that MLL-ENL target genes stood out with a supraphysiological accumulation of H2BUB accompanied by hyper-accelerated transcription rates. Interestingly, examination of Wilms tumor specific ENL mutants allowed to elucidate the underlying mechanism of the MLL-fusion induced ENL hyperactivity. Introduction of Wilms-specified ENL-mutating substitutions into primary hematopoietic cells induced aberrant transcription and H2BUB modification of Hoxa9 and Meis1, two sentinel loci for polycomb action. This was dependent on the conserved YEATS domain of ENL that operated as “switch” binding either histone H3 or PAF1 thus effectively regulating ENL function as anti-repressor or elongation factor, respectively. With the use of p53-deficient cells in combination with PAF1 and thus perturbed proper silencing. This effect was intensified in an MLL-ENL fusion where MLL itself provided a constitutive tether to PAF1 effectively creating a “super-transcription factor” that constitutively combined anti-repression with elongation capabilities.

Summary/Conclusions: In summary, targeting histone ubiquitination may be an additional Achilles heel for mixed lineage leukemia that merits further investigation of therapeutic utility.

P528

PKC EPSILON SUPPORTS ACUTE MYELOID LEUKEMIA BY MAINTAINING MITOCHONDRIAL REDOX HOMEOSTASIS

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Background: Although numerous genetic mutations contribute to the etiology and pathophysiology of acute myeloid leukemia (AML), the molecular machinery that is not mutated but supports AML biology remains largely unknown. Several studies have shown that AML cells, irrespective of genetic sub-type, display an oxidized intracellular redox environment compared to their healthy counterparts. The redox environment of AML cells is largely due to the elevated superoxides and peroxides display an oxidized intracellular redox environment compared to their healthy counterparts. The redox environment of AML cells is largely due to the elevated superoxides and peroxides of human and murine AML cell lines.

Methods: FACS-based purification of shRNA-expressing cells followed either by: 1) using nano LC-MS/MS. Annexin-V staining was analyzed by flow cytometry to monitor cell death. As a result, ROS homeostasis is tightly regulated ENL-fusion of function assays. Cytoplasmic and mitochondrial superoxides and peroxides of human and murine AML cell lines in vitro and in vivo were measured using nano LC-MS/MS. Annexin-V staining was analyzed by flow cytometry to monitor cell death.

Results: We have discovered that inhibition of PKCε: 1) promoted the death of leukemic cell lines in vitro, 2) AML cell lines in vivo and 3) obstructed the growth of 5 out of 7 PD-AML samples in vitro. At the molecular level, we observed that PKCε inhibition led to a significant and dose-dependent increase in mitochondrial-produced superoxides—a specific type of ROS. Moreover, we found that enforced expression of PKCε can protect AML cells from lethal effects of superoxide-inducing agents 2-thienyltrifluoroacetone and Antimycin A. To identify potential ROS-regulatory enzymes downstream of PKCε, we performed whole cell proteomics and found that the mitochondrial superoxide-neutralizing enzyme SOD2 is decreased in AML cells depleted of PKCε. Similar to PKCε inhibition, we also observed increased inhibition of SOD2 reduced the expansion of AML cell lines and PD-AMLS in vitro as well as significantly extended the onset of MLL-AF9-driven AML in vivo (p=0.0042). Finally, we also found that enforced expression of SOD2 in tandem with another anti-oxidant enzyme Catalase, reverses the anti-leukemia effects of PKCε inhibition confirming that PKCε supports AML pathophysiology by maintaining mitochondrial redox homeostasis.

Summary/Conclusions: Our results indicate that PKCε and SOD2 regulate mitochondrial redox homeostasis to support AML cell survival and disease progression and thus may represent a foundation for designing and developing novel therapeutic strategies.

P529

Abstract withdrawn.

P530

ROLE OF SHP2 IN A MOUSE MODEL OF AML CARRYING FLT3-ITD ALONG WITH LOSS OF TET2

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Background: SHP2, a protein tyrosine phosphatase coded by Ptnp11, is an essential protein that integrates signals from several different tyrosine kinase receptors with all the major intracellular signaling pathways such as ERK, PI3K and STAT pathways and regulates cell survival, proliferation and differentiation. One of the SHP2 dependent cytokine receptor kinase, FLT3 when mutated to constitutively activated co-operators with other genetic lesions like loss of Tet2 and Dnmt3a leading to transformation of myeloproliferative neoplasm (MPN) to acute myeloid leukemia (AML) in mouse models. Tet2 and Dnmt3a are involved in regulation hematopoietic stem cell (HSC) self-renewal and differentiation programs through regulation of DNA methylation. One of the many themselves to LS of MPN but when present in combination, leads to AML. These mouse models of AML have a more pronounced stem cell phenotype. Our studies have shown that they are resistant to traditional chemotherapy with or FLT3 targeted kinase inhibitor. Aims: Inhibition of SHP2 catalytic activity by a small molecule allosteric inhibitor has been recently demonstrated to retard the growth of receptor tyrosine kinase driven malignancies. Therefore, we wanted to investigate the role of SHP2 in leukemogenesis driven by loss of an epigenetic regulator (Tet2) and aberrant cytokine receptor tyrosine kinase (Flt3-ITD) signaling.

Methods: Mice were intercrossed to generate Ptnp11F/Te2F/Flt3ITD/+Mx1Cre+ or Ptnp11F/Te2F/Flt3ITD/+Mx1Cre mice. Deletion of Ptnp11 was induced between 8-10 weeks of age by injecting poly IC and changes in the hematopoietic compartment were analyzed by flow cytometry. Cell autonomous and non-autonomous effects of Ptnp11 on leukemogenesis were also evaluated in transplantation models.

Results: After ploy IC induced deletion of Ptnp11 there was a significant difference in the median survival between leukemia mice with deletion of Ptnp11 versus non-deleted (n=3). Though the Ptnp11 deleted leukemia mice showed almost complete loss of long term HSC with concomitant increase in short term proliferating HSC in the bone marrow, they were still able to home and engraft in lethally irradiated recipient mice. These results indicate that loss of Ptnp11 does not impair the engraftment of leukemic stem cells though in normal mice deletion of Ptnp11 impaired the ability to stem cells to home to bone marrow niche and engraft. Deletion of Ptnp11 in both primary mice and secondary recipients was also associated with deregulation of myeloid and lymphoid cell distribution both in the periphery and bone marrow. Mice with deletion of Ptnp11 in the context of Flt3ITD did not generate immature or mature B cells. The effects of Ptnp11 deletion were more severe in an AML model as compared to mice that received Ptnp11 deleted cells or when Ptnp11 was deleted after transplantation suggesting a role for SHP2 function in the bone marrow microenvironment in this model of leukemogenesis.

Summary/Conclusions: SHP2 has been recognized as a proto-oncogene on the basis of its ability to induce hematological malignancies when it is constitutively active and loss of SHP2 catalytic activity is associated with inhibition of tyrosine kinase driven malignancies. Our results demonstrate that the role of SHP2 in AML is dependent upon the presence of other genetic mutations. SHP2 regulates AML with loss of Tet2 with concomitant expression of Flt3-ITD through influence on both leukemic cells and the bone marrow microenvironment.

P531

CLUSTER REGULATION OF RUNX FAMILY BY “GENE SWITCH” TRIGGERS A PROFOUND TUMOR REGRESSION OF DIVERSE ORIGINS

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22nd Congress of the European Hematology Association
Background: Although Run-related transcription factor 1 (RUNX1) has been generally considered to be a tumor suppressor, a growing body of evidence suggests its pro-oncogenic property in acute myeloid leukemia (AML).

Aims: Demonstrate the antitumor potential of cluster regulation of RUNX with a "gene-switch" in AML as well as in dismal-prognostic solid tumors arising from diverse origins in vivo.

Methods: To assess the effect of RUNX-inhibition in AML cells, we performed series of shRNA-mediated RUNX knockdown experiments. To achieve cluster regulations in AML cells, we have computationally designed an agent which could irreversibly block the RUNX cluster genes expression profiling through dismantling protein-DNA interactions sequence-specifically (CRoX-1).

Results: Firstly, shRNA-mediated silencing of RUNX1 stimulated cell cycle arrest at G0/G1 phase and induced apoptosis in AML cells bearing wild-type p53. Besides, RUNX1 depletion induced remarkable induction of p53 as well as its target gene products and additive knockdown of p53 in these cell lines reverted the phenotype of RUNX1-depletion, indicating that RUNX1 is functionally dependent on proficient p53 pathway. In addition, cycloheximide chase assay revealed that RUNX1 negatively regulates the protein stability of p53 in AML cells. To assess data analysis and ChIP-seq experiments together with series of knockdown and restore experiments identified BCL1A and TRIM24 as critical mediators of p53 pathway activation in RUNX1-inhibited AML cells. Though RUNX1-depleted AML cells exhibited drastically slowed proliferation rate, a small sub-population of leukemia cells retained the proliferation potential even after silencing of RUNX1. Analysis of these residual AML cells revealed the reciprocal up-regulation of RUNX2 and RUNX3 expressions, suggesting that RUNX2 and RUNX3 might compensate for the loss of RUNX1 functions. As expected, additional knockdown of RUNX2 and RUNX3 in RUNX1-depleted AML cells effectively suppressed their proliferations. Thus the simultaneous targeting of RUNX clusters members as a cluster provides more stringent control of leukemia cells. Finally, we examined the antitumor potency of CRoX-1-mediated cluster regulations of RUNX. CRoX-1 treatment was indeed highly effective against leukemia as well as dismal-prognostic solid tumors arising from diverse origins in vitro. Moreover, this reagent was exceptionally well-tolerated in mice and exhibited excellent efficacy against xenograft mice models of AML, acute lymphoblastic leukemia, lung and gastric cancers, extending their overall survival periods in vivo. Since RUNX1 families take part in diverse physiologic functions not only in AML cells but also in normal hematopoietic cells and in various other vital organ tissues, we might expect criticisms in targeting whole RUNX family that it could trigger undesirable side-effects in vivo. Intriguingly, however, our drug was well-tolerated in vivo and through these experiments, we have coincidently found that the amount of total RUNX expressions was consistently higher in malignant tissues compared to their normal counterpart partners, and we believe that this gap offers pharmacological window to be targeted in clinical trials. Furthermore, we found that higher expressions of estimated total RUNX amount demarcate significantly poorer-prognosis patient cohorts in a wide variety of cancers, underpinning the rationality of RUNX-inhibition strategies in cancer treatment.

Summary/Conclusions: This work identified the crucial role of RUNX cluster in the maintenance and the progression of cancer cells, and the indicated gene switch technology-dependent its modulation would be a novel strategy to control malignancies.

P532
PHOSPHOPROTEOMICS AND MASS CYTOMETRY SIGNATURES OF PRIMARY AML CELL DIFFERENTIATION ARE ASSOCIATED WITH SENSITIVITY TO KINASE INHIBITORS
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1Cancer Dept., Haematologic Oncology, 2Flow Cytometry Core Facility, 3Tissue Bank, Barts Cancer Institute, 4Haematological Medicine, King’s College London School of Medicine, London, United Kingdom

Background: Kinase signalling is frequently deregulated in cancer cells. In the case of AML, the high recurrence of activating mutations in kinases and other kinase signalling regulators including FLT3 and RAS has stimulated the investigation of treatments based on kinase inhibitors. The success of kinase inhibitors depends on an accurate stratification of patients into response groups. The impact of genetic mutations on the sensitivity of primary AML to kinase inhibitors remains poorly defined and these have not been translated into effective therapies. The activity of a kinase can be affected by factors other than gene mutations and the sensitivity of leukemic cells to kinase inhibition depends not only on the activity of the targeted kinase. Thus, the integrative analysis of different biochemical features could improve the implementation of precision medicine therapies based on kinase inhibitors.

Aims: By the integration of multiple omics approaches, we aimed to generate molecular signatures, which can rationalize why some primary AML cells are resistant to treatment with different kinase inhibitors while others are sensitive to the same treatments.

Methods: In this investigation, we used a multomics approach to stratify 36 AML biopsies as a function of their cellular sensitivity to "ex vivo" treatment with TAK-715, silmitasertib, PF03758309, midostaurin and trametinib, which target p38, and/or PI3K and MEK, respectively. The same samples were analysed using different omics platforms: (i) mass spectrometry for phosphoproteomics, proteomics and kinomic profiling, (ii) mass cytometry for immunophenotyping and (iii) next generation sequencing for mutational profiling.

Results: Our integrative analysis identified two independent signatures that stratified our cohort of patients in sets of differentiated and undifferentiated cases. The phosphoproteomics signature divided our set of AML cases in the M1-like and M4-like groups (Figure 1A). The mass cytometry signature, which represented myelomonocytic markers that were co-expressed at the cell surface, split our cohort of patient in the CD56+ and CD56− groups. Remarkably, the M4-like and CD56− groups representing the non-differentiated cases, as well as the M1-like and CD56+ groups representing the non-differentiated cases, showed a high degree of overlap. Differentiated groups over-phosphorylated 3 times as many proteins as the non-differentiated groups, including kinases at sites linked to their activity. Mutations in genes involved in kinase signalling were also more frequent in differentiated cases. Kinase activity analysis using KSEA estimator that differentiated groups presented an enriched activity for PAK, MEK, ERK or PKC. Ontology analysis showed that non-differentiated cells over-phosphorylated nuclear proteins with DNA binding properties, while the differentiated cells increased the phosphorylation of membrane and cytoplasmic proteins linked to the small GTPase signalling. More interestingly, cases in differentiated groups were more sensitive to PF03758309, trametinib and midostaurin than those in the non-differentiated sets (Figure 1B for groups defined by the phosphoproteomics signature). Finally, differentiated cases as defined by the mass cytometry signature in our cohort of patients, or by a CD marker mRNA expression signature in the ATCG database, presented with significantly reduced survival when compared to the groups of non-differentiated cases.

Summary/Conclusions: Our data indicate that differentiated cells activate pro-survival kinases like PAK, PKCD or MEK which make them more sensitive to the inhibitors PF03758309, midostaurin or the FDA-approving drug trametinib. Since patients with differentiated cells present a reduced overall survival, treatment with these compounds may benefit patients in this higher risk group.

P533
CLINICAL IMPACT OF TET2 MUTATIONS IN ACUTE MYELOID LEUKEMIA PATIENTS HARBORING CEBPA MUTATIONS: A STUDY OF THE AML STUPY (GRAN ALSG)
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haematologica | 2017; 102(s2) | 203
SRSF2

TET2

Summary/Conclusions:

also see an increased expression of ROS in Gfi1b deficient leukemic cells, a higher activity of the FOXO pathway as well as reduced p38 activity. The combination of these findings contributes to the higher number of leukemic stem cells in Gfi1b deficient leukemic cells. To reduce the high level of ROS in leukemic stem cells we use with N-Acetylcysteine (NAC). Use of NAC impeded growth of Gfi1b deficient cells in vitro.

Conclusion/Conclusions: Gfi1b act as a tumour suppressor by restricting number of leukemic stem cells and treatment with NAC opens a potential targeted therapy for AML patients with low/absent expression of Gfi1b.

P535

VARIANTE ALLELE FREQUENCY KINETICS OF TYROSINE KINASE GENE MUTATIONS IN CORE-BINDING FACTOR ACUTE MYELOID LEUKEMIA MUTATIONS IN CORE-BINDING FACTOR ACUTE MYELOID LEUKEMIA (AML) MUTATIONS IN CORE-BINDING FACTOR ACUTE MYELOID LEUKEMIA (AML) (CBF-AML) patients. The aim of our study was to investigate the frequency of tyrosine kinase gene mutations in CBF-AML patients. Three different tyrosine kinase genes (KIT, PDGFRA, and NRAS) were investigated in 80 CBF-AML patients. The frequency of KIT mutations was 4.1%, PDGFRA mutations 5.1%, and NRAS mutations 2.7%. The frequency of tyrosine kinase gene mutations was significantly higher in patients with higher risk cytogenetics. The frequency of tyrosine kinase gene mutations was significantly higher in patients with higher risk cytogenetics. The frequency of tyrosine kinase gene mutations was significantly higher in patients with higher risk cytogenetics. The frequency of tyrosine kinase gene mutations was significantly higher in patients with higher risk cytogenetics.

Results: In total200 AML pts (age 18 to 78 years) with

TET2

WT

and conventional chemotherapy, and ii) to conduct gene set enrichment analyses.

Methods: Whole-exome-sequencing (WES) was performed in paired diagnosis, remission and relapse samples of 38 patients with CBF-AML [16(1); and t(8;21)-

Aims: To characterize clonal evolution in paired samples obtained at diagnosis, remission, and relapse by using matched whole-exome sequencing.

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diagnosis. 8.9 variants per patient were found as compared to 5.7 at relapse. 52% variants were present at diagnosis, 26% at relapse only, and 22% were present at both, diagnosis and relapse. With regard to the most commonly altered signaling genes KIT and NRAS we found the following pattern: The median VAF at diagnosis was 23% and 26% for KIT and NRAS, respectively. Of note, the initial KIT and NRAS clone was lost (VAF <5%) in 71% (exon 17, n=9; exon 8, n=2; exon 11, n=1) and 100% of cases (exon 2, n=5; exon 3, n=3). Comparing the VAF kinetics between patients treated with and without dasatinib, baseline KIT mutations became subclonal (VAF <5%) in all patients receiving dasatinib (n=8), whereas they were still detectable in 4/6 (67%) patients who were intensively treated without the addition of dasatinib. NRAS became subclonal (n=8) irrespective of the treatment regimen. In one KIT mutated patient treated with dasatinib the baseline KITD816V mutation (exon 17) was lost at the time of relapse, but a KITD819V mutation (exon 8) was acquired instead. Gene set enrichment analyses revealed different mutation signatures at diagnosis and relapse: At diagnosis, there was a significant enrichment for genes associated with MYC overexpression. Variants that were recurrently present at diagnosis and relapse showed enrichment for genes affected in KRAS overexpression models. Relapse samples were additionally enriched for gene variants involved in the mitotic spindle assembly.

Summary/Conclusions: Differences in the allelic composition were found between diagnosis and relapse regardless of the CBF-AML subtype. Our data suggest that the KIT clone might be successfully eradicated under dasatinib treatment whereas persistence of KIT mutant clones was more commonly seen under conventional chemotherapy. The frequent loss of KIT and NRAS mutations during therapy suggests that relapse is triggered by alternative genetic lesions. Relapsed disease may represent a distinct biology which is characterized by mutations that cluster in different pathways. Further analyses are ongoing including study cohort expansion, as well as inclusion of RNA sequencing results.

Acute myeloid leukemia - Biology 4

P536
P38B MAPK INTERACTS WITH SET REGULATING ITS INHIBITORY EFFECT ON PP2A ACTIVITY IN ACUTE MYELOID LEUKEMIA

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Background: Despite improvements in our understanding of the molecular evolution of acute myeloid leukemia (AML), the overall cure rates remain low, and most patients die from the disease despite achieving initial remission upon treatment. It is therefore necessary to open new therapeutic perspectives aimed at molecular targets. PP2A phosphatase inactivation is a recurrent event in hematological tumors. Our group has reported that SET, an endogenous inhibitor of PP2A, is overexpressed in 28% of patients with AML. Furthermore, the oncogenic activity of PP2A activating drugs (PADs) depends on the interaction/sequestration of SET, pointing out the significance of this oncogene in AML. Drug inhibition of several MAPKs in AML cell lines showed that only p38 inhibitors activate PP2A and decrease SET protein.

Aims: Therefore, we hypothesized that p38 could regulate SET at posttranslational level, leading to PP2A inactivation.

Methods: AML cell lines and primary human samples were analyzed by western blot, immunoprecipitation, immunofluorescence, treatment with pharmacological inhibitors and siRNAs. Phosphorylation assays by in vitro kinase assay with recombinant proteins were performed.

Results: Knockdown of the two major isoforms of p38-MAPK, p38α and p38β, demonstrated that only p38β was able to reduce SET protein levels and increase PP2A activity. To decipher this mechanism of action, we performed protein immunoprecipitation and immunofluorescence in the AML cell lines HL-60 and MOLM-13. p38β co-localized and bound to SET mostly in the cytoplasm stabilizing it, since treatment with ciclohexymide in the absence of p38β induced SET degradation. The stabilization role was in coordination with SETBP1, which co-localized with both SET and p38β. Interestingly, 12 out of 14 AML cell lines tested showed high expression of p38β protein levels in comparison to p38α, as well as 5 out of 7 AML primary patient samples. Furthermore, expression analysis in a large series of adult de novo AML cases previously reported (Cancer Genome Atlas Research Network, 2013) showed a positive correlation between p38β (MAPK11) and SET (R²=0.416, p<0.001), but not between p38α and SET. We and others have shown that PADs retain SET in the nucleus. Our results showed that p38 phosphorylates SET not directly, but through the activation of casein kinase 2 (CK2), leading to the retention of SET in the nucleus and, therefore, contributing to the inactivation of PP2A in AML cells. Of note, CK2 is overexpressed in both AML cell lines and patient samples.

Summary/Conclusions: p38 is able to activate CK2 which phosphorylates SET and, as consequence, facilities its trafficking to the cytoplasm, contributing to PP2A inactivation in AML cells. Moreover, p38β binds to SET in the cytoplasm, contributing to its stability and leading to PP2A inactivation. In this regard, we have preliminary evidences that combination therapy with PADs and the CK2 inhibitor CX4945 reduces significantly the viability of AML cells, supporting that novel treatment modalities that can target multiple components of the same pathway may help to achieve a more sustained therapeutic benefit.

P537
GENETIC LANDSCAPE OF ACUTE ERYTHROID LEUKEMIA

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Background: Acute erythroid leukemia (AEL) is a unique subtype of acute myeloid leukemia (AML) characterized by the predominance of erythroid components with increased ring sideroblasts as well as frequent myelodyplasia. However, due to its rarity, the molecular pathogenesis of AEL has not fully been elucidated, except for frequent TP53 mutations.

Aims: This study was designed to clarify the mutation profile of AEL distinct from other AML. Additionally, we aimed at developing a molecular classification scheme for AEL.

Methods: We performed a comprehensive genetic study, in which paired tumor/normal DNA from 22 AEL cases were analyzed using whole exome sequencing (WES). Whole-exome sequencing data from 3 AELs generated by The Cancer Genome Atlas (TCGA) was also included in the analysis. Subsequently, a total of 84 AEL cases were screened for mutations in 67 driver genes associated with myeloid malignancies using targeted-capture sequencing, in which RNA bait sets were also designed for a total of ~1150 single nucleotide polymorphism sites to allow for genome wide copy number abnormalities and other allelic imbalances.

Results: Median age at diagnosis was 58.5 (21-87) years old. Among the 77 patients with clinical information available, 62 patients were diagnosed with de novo AML, 13 with secondary AML, and 2 with treatment-related AML. On average, 18.4 and 3.4 mutations were detected per sample in whole-exome and target-sequencing when compared to AML, respectively. Both platforms being combined, most frequently observed was TP53 mutations (n=26, 31%) with complex karyotype being accompanied in most cases (25 cases), which were associated with a significantly shorter overall survival (P<0.001). Other frequently mutated genes were those encoding major components of the cohesin complex, including STAG2 (12%), SMC1A (4.8%) and RAD21 (2.4%), which were mutated in as high as 30% of the cases. The splicing machinery (18%) and epigenetic regulators (45%) were also some of the main targets of mutations, including SRSF2 (12%), U2AF1 (4.8%), WT1 (15%), TE2 (19%) and IDH1/2 (2%). TP53 mutations were mutually exclusive with cohesin mutations (p<0.01) and those in splicing machinery (p<0.05). In addition, mutations in PTDs were less frequent in AEL, compared to de novo AML and MDS. The frequency of these mutations was not statistically different between de novo AEL and secondary AEL.

Summary/Conclusions: WES and follow-up targeted-capture sequencing revealed a landscape of mutations in AEL. Frequent mutations in TP53, splicing factors, the cohesin complex, and epigenetic regulators were characteristic of AEL and thought to be involved in its pathophysiology. Mutations in TP53 defined a subgroup with distinct genetic and prognostic features. Our result indicated a similarity between AEL and high-risk MDS/secondary AML, supporting the recent revision of the WHO classifications, in which AEL was reclassified into MDS and AML not otherwise specified.

P538

THE MOLECULAR LANDSCAPE OF MLL-PTD AML: SPECIFIC CONCURRENT MUTATIONS, CLINICAL OUTCOME AND GENE EXPRESSION SIGNATURES

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Background: Partial tandem duplications (PTDs) in the Mixed Lineage Leukemia (MLL) gene, currently known as Lysine Methyltransferase 2A (KMT2A) are acquired in-frame internal duplications present in 5–11% of acute myeloid leukemia (AML). MLL-PTDs are predominantly present in cytogenetic normal AML and occasionally in AML with trisomy of chromosome 11. MLL-PTD cases are known as a poor prognosis marker in AML.

Aims: Evaluate the mutational landscape, prognostic value and gene expression signatures of MLL-PTD AMLs in comparison to a well-characterized AML cohort without MLL-PTD.

Methods: cDNA of 2310 AML patients enrolled in the adult HOVON-SAKK clinical trials (from 1995 to 2013) were analyzed for the presence of an MLL-PTD. Mutational screening based on next generation sequencing (NGS) was performed using the Illumina TruSight Myeloid panel on the Illumina HiSeq2500. An independent cohort of 632 de novo AML patients without MLL-PTD served as control. The gene expression profiling was assessed of all AML cases using Affymetrix HuGene1.0stPlus2.0 GeneChips as previously described (Verhaak et al., 2009).

Results: MLL-PTD was detected in 118 (5.1%) out of 2310 AML patients. MLL-PTD were significantly associated with trisomy 11: 7% vs 1% (p=0.0037), normal karyotype: 65% vs 53% (p=0.0102) and complex karyotype: 1% vs 14% (p=0.0002). All patients with MLL-PTD harbored translocations involving MLL and coexpression of AML1 and MLL fusion genes. The targeted NGS was performed on 87 out of 118 patients due to availability of gDNA. The number of mutation detected in MLL-PTD AMLs ranged from 0–6 mutations with an average of 3 mutations per patient. The most frequently mutated genes in MLL-PTD AMLs were DNM3TA, FLT3, R AI1, RUNX1, IDH1, and TET2. In the context of the 632 AMLs without MLL-PTD mutations in several genes appeared to be significantly associated with the MLL-PTD, i.e., FLT3-ITD: 34% vs 13% (p=0.0001), IDH1: 16% vs 9% (p=0.0133), U2AF1: 9% vs 3% (p=0.0158) and IDH2: 23% vs 12% (p=0.0181) or inversely associated, i.e., NPM1: 3% vs 23% (p=0.0001), NRAS: 5% vs 22% (p=0.0002) and TP53: 3% vs 10% (p=0.0487). Overall, the numbers of mutations in the spliceosome (p=0.03), tumor suppressor (p=0.0388), and transcription factor genes (p=0.0408) were significantly higher in MLL-PTD AMLs compared to MLL wild-type AMLs. As expected, the MLL-PTD appeared to be significantly associated with inferior outcome (MLL-PTD (n=74) and without MLL-PTD (n=1764); OS, p=0.05). Association of the presence of an MLL-PTD with EFS was only borderline significant (p=0.07). Within MLL-PTD AML, DNM3TA mutations are associated with inferior overall survival (HR: 2.06; 95%CI: 1.19-3.58; p=0.010). Although low numbers, MLL-PTD AML patients that harbor NRAS mutations do even worse (HR: 6.54; 95%CI: 2.45-17.49; p<0.001. In multivariate analysis both markers remain significant when combined with WBC counts and cytogenetics. Multiple homeobox-related gene family members were overexpressed in MLL-PTD AML. The top-35 differentially expressed genes included HOXB5, HOXB6, HOXB7, HOXB8, HOXB9 and NKX2.3. In an association model, which takes all other known subsets of AML into account, other HOX-related genes, such as HOXAT7, HOXAX9 and NKX2.5, seem to be most consistently overexpressed in MLL-PTD AML. In contrast, these specific gene expression changes were absent in AML with translocations involving MLL on 11q23. Additional analyses in AML subsets based on the current mutations will be carried out to investigate whether these are limited to MLL-PTD AML molecular subtypes.

Summary/Conclusions: MLL-PTD AML carries specific gene expression signatures and specific subsets of concurrent mutations with clinical value.

P539

EXPLORING THE IMPACT OF LOSS OF FUNCTION STAG2 MUTATIONS ON CHROMATIN ARCHITECTURE IN MDS/AML

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Background: The Cohesin complex is an evolutionarily conserved multimeric protein complex that maintains the structure of chromatin and is required for transcriptional and epigenetic remodeling. STAG2 functions within the cohesin complex to maintain the architecture of the genome.

Aims: To explore the impact of a loss of function STAG2 mutation on the chromatin architecture within an isogenic cell based model.

Methods: Using an CRISPR generated isogenic model we have investigated the impact loss of STAG2 has on the chromatin architecture of a hematopoietic environment. Genome wide binding profiles for STAG1, STAG2 and CTCF were generated using ChiP-seq to elucidate areas of differential between STAG members. In addition, binding profiles for H3K27ac, H3K27me3 and H3K4me1 were generated using ChiP-seq to provide genome wide identification of active and repressed enhancer regions, with the regions ranked to identify both normal and super-enhancer regions. These samples were then compared to ATAC-Seq profiling of open and closed chromatin regions as well as RNA-seq samples to provide information on gene activity in relation to chromatin state in the absence of STAG2.

Results: Our results indicate that STAG1 binding profiles after following loss of function of STAG2, with an increase in binding peaks from ~17,000 to 25,000, however several sites identified by ChiP-seq are not compensated for. Histone mark profiling identified widespread expansion of the H3K27ac mark and a decrease in regions of H3K27me3 consistent with loss of boundaries within topologically associated domains. This spread of an activator mark correlates with altered gene expression and the changes observed in ATAC-seq profiling of altered chromatin accessibility. The open chromatin regions identified through chromatin capture-seq aligned with Type II-soldiers. RuBNF1 expression was maintained in the absence of STAG2.

Summary/Conclusions: This research into the aberrant and non-canonical function of STAG2 in the spatio-temporal genomic architecture in hematological malignancies and begins to yield insight into the clinical implications of mutations within the cohesin complex.
Background: Mutations involving the MLL gene at 11q23 are found in 10% of adult and 18% of childhood acute myeloid leukemia (AML) cases. The most frequently occurring MLL mutations are chromosome translocations that fuse the MLL gene in-frame with a second partner gene, creating novel fusion proteins (MLL-FPs). MLL-AF9 is the most common MLL-FF in AML. Despite much progress in the overall management of AML, patients carrying MLL-rearrangements still have a poor survival prognosis and limited response to existing therapy. This is in part due to the low therapeutic indices and narrow therapeutic windows of current chemotherapeutic agents, therefore underscoring the need to develop improved, targeted therapies. MYB is a direct downstream target of MLL-AF9. Recent studies indicate that MLL-AF9 leukemia cells are more affected by MYB knockdown compared to normal hematopoietic stem progenitor cells. This is despite the fact that MYB is known to be essential for the establishment of definitive hematopoiesis. This suggests that a therapeutic window may be achieved through targeting MYB. Therefore, by understanding more about the role of MYB in MLL-AF9 leukemia and the network it regulates, we may able to exploit this knowledge to target MYB directly by interfering with its function or indirectly via its downstream targets.

Aims: To understand the molecular function of MYB in MLL-AF9 leukemia.

Methods: We performed genome-wide MLL, MLL-AF9, H3K27ac, H3K4me3 and H3K4me1 chromatin immunoprecipitation sequencing (ChIP-seq) and Assay for Transposable-Accessible Chromatin with high throughput sequencing (ATAC-seq) in two MLL-AF9 leukemia models to identify putative regulatory regions of MYB and those of a direct MYB gene target, BCL2. The chromatin conformation capture technique, Capture-C (one vs all) was used to further characterize interactions from the MYB promoter. We then performed siRNA knockdown of MYB and assessed the effect of MYB loss on its downstream druggable target BCL2, using RT qPCR, Western blotting and ChIP qPCR.

Results: We identified MLL-AF9 binding to novel putative enhancers of MYB as defined by regions co-bound by H3K27ac, H3K4me1 and marked by open chromatin on ATAC-seq. Furthermore, Capture-C from the MYB promoter identified novel putative enhancer-promoter interacting domains 100-200kb apart that are co-bound by MYB but not MLL-AF9. This suggests long-range autoregulation of MYB. Next, siRNA knockdown of MYB results in loss of MYB binding at the BCL2 promoter and its downstream enhancer by ChIP qPCR. There is a corresponding loss of BCL2 mRNA and protein expression in MYB knockdown cells compared with control, confirming that BCL2 is directly regulated by MYB.

Summary/Conclusions: We have identified for the first time, regulation of MYB by MLL-AF9 via putative enhancers, and also an autoregulatory role of MYB involving long-range cis-interactions. Furthermore, we confirm that BCL2 is directly regulated by MYB in MLL-AF9 leukemia, suggesting a molecular rational for using BCL2 inhibitors in MLL-AF9 leukemia therapy.

CD123-SPECIFIC CHIMERIC ANTIGEN RECEPTOR T-CELL THERAPY IN ACUTE MYELOID LEUKAEMIA

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Background: Acute myeloid leukemia (AML) is a heterogeneous disease characterized by clonal evolution of myeloid precursors in bone marrow and peripheral blood resulting in accumulation of leukemic blasts and severe impairment of normal hematopoiesis. Despite advances in our understanding of AML biology, development of novel therapies has been limited with 43% relapse rate and 18% of patients never attaining clinical remission (CR) with frontline induction treatment. Chimeric antigen receptor (CARs) T cells specific for tumour-associated antigens are emerging to be an effective form of immunotherapy for AML. A small number of in vitro and in vivo studies have evaluated the efficacy and specificity of CAR T cell immunotherapy in AML by targeting interleukin three receptor alpha (IL3RA, CD123), a molecule over-expressed on AML blasts and leukemia stem cells (LSC) compared to normal haematopoietic stem cells (HSCs).

Aims: In this study, we investigated the efficacy of a second generation CAR expressing six single-chain variable fragments (scFv) with different affinities for CD123 and evaluated the cytotoxic effect of different co-stimulatory domains (CD28 versus 41BB) using a co-culture assay. Furthermore, we also evaluated the cytotoxic effects of a dual targeting CAR (against CD123 and CD33) using the same assay conditions.

Methods: Six lentiviral constructs (two high, two moderate & two low affinity) were transduced (MOI 1:5) into peripheral blood mononuclear cells (PBMCs) from healthy donors and their cytotoxicity was examined by flowcytometry on leukemic cell lines; KG1 (CD123+, CD34+, CD33+), K562 (CD123+, CD34+, CD33+) and AML mononuclear cells (MNCs).

Results: Flowcytometric analysis confirmed the expansion of T cells from PBMCs and the cytotoxicity of the six CARCD123 constructs against CD123+ve cells. The high affinity CARCD123 (4nM kD & 4nM kD K136Q) T cells demonstrated enhanced cytotoxicity compared to moderate (56nM kD, 56nM kD A105G) and low affinity (10nM kD, 10nM kD V242G) CARCD123 in both leukemic cell lines and also in alligenic AML MNCs. Both the highest affinity CARCD123 constructs were also tested in cell lines using increasing effector: target ratios (1:2, 1:4 & 1:10) displaying consistent cytotoxicity and were also effective against autologous AML MNCs (target cells) and PBMCs (effector cells) from two patients. T cell activation was confirmed by ELISA and showed increased IFN-γ (500-2000 fold) and TNF-a (150-200 fold) levels. Previous studies have confirmed the distinction in CAR efficiency using CD28 versus 41BB co-stimulatory domains; CD28 co-stimulation augmented, whereas 4-1BB co-stimulation reduced T cell exhaustion induced by continuous CAR signaling. To confirm persistence of the CAR cytotoxicity, we constructed a high affinity CAR substituting CD28 with a 4-1BB co-stimulatory domain and obtained similar cytotoxicity results on K562 and U937 cell lines. Furthermore, a novel dual targeting CAR in which the activation domain (CD3ζ) is directed against CD33 and the costimulatory domain (CD28) directed against CD123 enhanced the specificity of the CAR towards leukemic cells; reducing “on-target but off-organ effects”. Results obtained in co-culture assay against KG1 [Fig:1b] and K562 cell lines [Fig:1c] with varying effector: target ratios were demonstrated results similar to the high affinity single targeting CAR.
We have recently discovered that FLT3-ITD+ AML cells are highly sensitive to the FDA-approved, senescent-AKT inhibitor palbociclib (IBRANCE by Pfizer). The effect is ascribed to the transcriptional activity of CDK6 on FLT3 and PIM1 - a feature not shared by CDK4.

**Aims:** FLT3-TKI treatment provides short-term disease control but relapse invariably occurs within months. Acquired resistance on FLT3-D835Y tyrosine kinase domain (TKD) signaling, clinical protipogetic processes and recurrence of the disease, and deliver new rationales for precision medicine approaches: still, whereas a comprehensive description of AML mutations and clonal architecture through the DNA damage response (n=50), or in immune-related processes (n=30). Panel we took advantage of the HaloPlex High Sensitivity (HS) technology, allowing a more precise definition of mutations and clonal architecture through sequencing was performed on an Illumina HiSeq2500n instrument. Variant calling was performed using a pipeline based on the freeBayes algorithm, and FLT3-ITD status was inferred using Pindel.

**Results:** Sequencing yielded uniform and consistent coverage of all target amplicons and a 612x mean depth-of-sequencing, resulting on average in 117 unique barcodes for each region. Among the 79 diagnosis samples we identified 293 mutations (204 of which by HaloPlex High Sensitivity (HS) technology, allowing a more precise definition of mutations and clonal architecture through sequencing was performed on an Illumina HiSeq2500n instrument. Variant calling was performed using a pipeline based on the freeBayes algorithm, and FLT3-ITD status was inferred using Pindel.

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with the Papaemmanuil dataset, we observed a weaker correlation for relapses after CT ($r^2=0.69$) and an even more marked deviation for post-transplant relapses ($r^2=0.45$). This difference was mainly explained by the enrichment in both relapse cohorts for FLT3-ITD (25% in diagnoses vs 55% and 48% at relapses after CT and allo-HSCT, $p <0.01$ for both comparisons) and WT1 mutations (5% vs 25% and 22%, $p <0.01$ for both comparisons). For 24 cases it was possible to longitudinally compare the mutational profile of AML at diagnosis and relapse in the same patient: we observed higher stability in relapses after CT, with 50% of cases carrying the same pattern of mutations present at diagnosis, whereas at relapses after allo-HSCT changes were more frequent, with 70% of patients displaying new gains or losses.

**Summary/Conclusions:** Taken together, our data evidence that the genomic landscape of AML at relapse can be significantly different from the one documented at diagnosis, suggesting that the selective pressure mediated not only by intensive chemotherapy, but also by the graft-versus-leukemia effect, can be potent drivers of clonal evolution. From the practical standpoint, the pattern of emergence of novel mutations that we documented should be taken into account not only for targeted salvage approaches, but also for the design of post-remission strategies aiming to prevent relapse.

**Abstract withdrawn.**
analyses, 81% of pts had de novo AML, 15% secondary AML, 3% therapy-related AML, and 2% high-risk MDS. Recurrent gene mutations in AML were studied from bone marrow aspirates or peripheral blood using a targeted leukemia genotyping assay covering 68 genes. We analyzed known mutual functional hotspots or the entire coding sequence of the genes by multiplexed amplicon sequencing (Agilent Technologies, mean target coverage of 460 x). We studied association of E-sel binding, evidence of on-target effect (reduction in sE-sel), lack of DLT at any dose level, and clinical outcomes. Ph1/Ph2 combined median age was 55yrs (range 26-84) with 70% male pts. Prior AML history included 26% primary refractory, 36% CR1<6 mos; 17% prior SCT; 52% unfavorable cytogenetics (by SWOG/S0607 criteria; 3% 3q/4 AEs were febrile neutropenia (36%), sepsis (26%), bacteremia (13%), hypoxia (13%). Oral mucositis (zG2) developed in 12% of pts. The 30 and 60 day mortality rates were 0 and 7%, respectively; induction mortality was 0%. The remission rate (CR/CRI) was 45% (19/42) with an ORR (CR/CRI/MLFS/PR) of 50% (21/42). Observed/expected remission rate (OS) was 24% (10/42). With a median follow-up of 11 mos, the Ph 1 median Disease Free Survival was not reached and Overall Survival was 7.6 mos. The median E-sel ligand expression at baseline was 35% (range, 1-75%) of blasts in the bone marrow, and was higher in those achieving remission.

Summary/Conclusions: The addition of GMI-1271, a novel E-sel antagonist, to MEC chemotherapy is well tolerated; oral mucositis, commonly severe with MEC, is observed at low severity in this study. Clinical outcomes include a high ORR and remission rate (CR/CRI), low induction mortality, and promising survival outcomes in pts with R/R AML. Furthermore, the baseline expression of E-sel in this population suggests relevance of the target and is predictive of response.

P547

GMI-1271, A POTENT E-SELECTIN ANTAGONIST, IN COMBINATION WITH CHEMOTHERAPY IN RELAPSED/REFRACTORY AML: A NOVEL, WELL-TOLERATED REGIMEN WITH A HIGH REMISSION RATE


Background: Expression of the adhesion molecule E-selectin (E-sel) in the vasculature of the bone marrow is associated with infiltrative disease, relapse, and poor survival in AML. GMI-1271 is a novel antagonist of E-sel that downregulates cell survival pathways and enhances chemotherapy response with improved survival compared to chemotherapy alone (Becker ASH 2013; Winick JS et al. 2014). Protection from common toxicities (neutropenia and mucositis) has been observed in preclinical models, also affording survival benefit (Winkler ASH 2013).

Aims: We assessed GMI-1271 plus salvage chemotherapy with mitoxantrone, etoposide, and cytarabine (MEC) for the treatment of patients (pts) with relapsed/refractory AML.

Methods: A Phase (Ph) 1 trial in pts with R/R AML escalated GMI-1271 across pharmacologically active doses from 5-200mg/kg combined with MEC to evaluate safety, tolerability and anti-leukemia activity. MEC consisted of mitoxantrone 10mg/m2, etoposide 100mg/m2, and cytarabine 1000mg/m2 IV for 5 cycles. MEC was dosed according to the National Comprehensive Cancer Network guidelines. Bone marrow aspirates were analyzed for E-sel ligand. Positive results were followed by a continuous 21-day cycle of GMI-1271 at 10mg/kg. A single dose of GMI-1271 was then added to the 21-day cycle. GMI-1271 was added at a 1-hour infusion, each composed of 3-6 patients. Treatment was administered as 1-hour daily infusion for 6 days. Abscesses were assessed by the treating physician as possibly/probably related to BST-236, all ‘on-target’ hematological toxicity events or bacterial infections derived from it.

Results: To date, 47 pts have enrolled (Ph 1=19; Ph 2=28 of planned 47). The recommended Ph 2 dose was 10mg/kg based on drug exposure, time over IC50 for E-sel binding, evidence of on-target effect (reduction in sE-sel), lack of DLT at any dose level, and clinical outcomes. Ph1/Ph2 combined median age was 55yrs (range 26-84) with 70% male pts. Prior AML history included 26% primary refractory, 36% CR1<6 mos; 17% prior SCT; 52% unfavorable cytogenetics (by SWOG/S0607 criteria; 3% 3q/4 AEs were febrile neutropenia (36%), sepsis (26%), bacteremia (13%), hypoxia (13%). Oral mucositis (zG2) developed in 12% of pts. The 30 and 60 day mortality rates were 0 and 7%, respectively; induction mortality was 0%. The remission rate (CR/CRI) was 45% (19/42) with an ORR (CR/CRI/MLFS/PR) of 50% (21/42). Observed/expected remission rate (OS) was 24% (10/42). With a median follow-up of 11 mos, the Ph 1 median Disease Free Survival was not reached and Overall Survival was 7.6 mos. The median E-sel ligand expression at baseline was 35% (range, 1-75%) of blasts in the bone marrow, and was higher in those achieving remission.

Summary/Conclusions: The addition of GMI-1271, a novel E-sel antagonist, to MEC chemotherapy is well tolerated; oral mucositis, commonly severe with MEC, is observed at low severity in this study. Clinical outcomes include a high ORR and remission rate (CR/CRI), low induction mortality, and promising survival outcomes in pts with R/R AML. Furthermore, the baseline expression of E-sel in this population suggests relevance of the target and is predictive of response.

P548

BST 236, A NOVEL CYTARABINE PRO-DRUG ALLOW, FOR THE FIRST TIME, THE DELIVERY OF HIGH CYTARABINE DOSES FOR OLDER OR REFRACTORY PATIENTS WITH ACUTE LEUKEMIA. RESULTS OF AN ONGOING PHASE III/II STUDY

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Background: Acute myeloid leukemia (AML) is associated with poor outcome in older patients and in patients unfit for standard induction therapy. Therapy of AML has not changed significantly since the 1970s and still relies on cytarabine as the first-line treatment. However, cytarabine therapy is associated with severe side effects, such as cerebellar toxicity, bone marrow suppression, and infections, leading to high treatment-related mortality rates. Hence, while the incidence of AML increases with age, advanced age and comorbidities may preclude the administration of intensive therapy altogether.

Aim: BST-236 (Astarbine) is a novel compound of cytarabine covalently bound to asparagine. It acts as a pro-drug of cytarabine, enabling delivery of high cytarabine doses to target cells with lower systemic exposure to the free drug and relative sparing of normal tissues. As such, BST-236 may serve as an ideal therapy for leukemia, particularly for delivering high doses of cytarabine to pts that are unfit or older. The aim of this study was to evaluate the safety and optimal dose of BST-236 in refractory/refractory-relapsed or newly-diagnosed AML patients unfit for standard induction therapy.

Methods: A Phase III/IIA prospective open label study enrolled adult relapsed/refractory or newly-diagnosed acute leukemia patients unfit for standard therapy. Patients are enrolled into 6 BST-236 escalating-dose cohorts (0.3-6 g/m2/d), each composed of 3-6 patients. Treatment was administered as 1-hour daily infusion for 6 days. Results: To date, treatment of cohorts 1-5 is completed, with 18 patients treated with up to 4.5 g/m2/day, median age 77 (27-90); 6 relapsed/refractory AML. The median age of newly diagnosed pts was 64 (27-81), and 12 patients were enrolled for standard chemotherapy (7 secondary AML; 8 de novo AML/ALL). The median age 79 (70-90). BST-236 treatment was well-tolerated. Only 6 SAEs in 4 cases were assessed by the treating physician as possibly/probably related to BST-236, all “on-target” hematological toxicity events or bacterial infections derived from it. Full blood and oropharyngeal or grade >2 typical cytarabine events such as gastrointestional, mucositis, or alopoeica were reported during BST-236 treatment or within 30 days of follow up. Response to the treatment was observed in 6 of the 12 newly-diagnosed patients, 4 of whom had a continuous complete remission (CR) and 2 had a partial remission (PR). The median overall survival (OS) of the newly-diagnosed pts was 3 months. The median OS of the newly-diagnosed non-responders was 2.5 months. No remission was reached in the 6 patients suffering from relapse or refractory AML and their median OS was 2.3 months.
Background: Genes are frequent genetic abnormalities in myeloid malignancies. The impact of the detection of such gene fusions is rising due to an increasing number of drugs targeting them as has been impressively shown for e.g. BCR-ABL1 and PML-RARA. Further, they can be used as biomarkers for disease monitoring.

Aims: Evaluation of targeted RNA sequencing for the detection of recurrent and novel fusion transcripts.

Methods: 102 cases with myeloid malignancies harboring 105 translocations identified by chromosome banding analysis were selected. Recurrent fusion genes had been confirmed by Fisher and/or RT-PCR. In cases with suspected novel fusions the rearrangement of one partner gene had been confirmed by Fisher. The following recurrent rearrangements identified by standard diagnostic procedures were present: PML-RARA (n=11), RUNX1-RUNX1T1 (n=7), CSFB-MYH11 (n=3), KMT2A-ELL (n=4), KMT2A-MLLT1 (n=4), KMT2A-MLLT10 (n=3), KMT2A-MLLT3 (n=3), KMT2A-MLLT4 (n=2), BCR-ABL1 (n=3), NUP98-NSD1 (n=3), DEK-NUP214 (n=1), and KAT6A-CREBBP (n=1). Further, cases harboring known fusions KMT2A-14A, RUNX1-21E1, ET6 (n=10), PDKFRB (n=10), RARA, NPM1 (n=2) and NUP98 (n=1) were included. Targeted RNA sequencing was performed using the TruSight RNA Fusion panel (Illumina, San Diego, CA) consisting of 7690 probes covering 507 genes known to be involved in gene fusions. Library was prepared according to manufacturer’s protocol with ~80ng RNA extracted from fresh/frozen samples. Sequencing was performed on the NextSeq instrument (Illumina) and analysis with the RNA-Seq Alignement App (BaseSpace Sequence Hub) using Star for Alignment and Manta for gene fusion calling with default parameters (illumina).

Results: In 42/45 (93%) cases with a recurrent rearrangement identified by standard diagnostics, RNA sequencing detected the respective fusion transcript. In addition, RNA sequencing was able to identify known and novel fusions in the remaining 57 cases. For KMT2A these were the following partner genes: MLLT1 (n=5), ELL (n=3), ITIF2, FLNC, ASXL2, DCP1B, MAML1 and ARHGEF12. Seven different partner genes were identified in RUNX1 translocations: PLAG1 (n=2), PRDM16, MECOM, ZFP26, MANIA2, NAMT2, and KIAA1549L. Five different partner genes were identified in ET6 rearranged cases: ABL1, CCDC126, ERG, FOXO1 and CFLAR-AS1. Most strikingly was the identification of the ET6-ET6VL fusion, which could not be suspected by cytofluorimetric analysis as the S ET6 FISH signal was located on chromosome 17. In 710 FDGF8R rearranged cases the partner genes were identified. These were WDR4, CCDC88C, MRIP, TNIP1, TPR, NF1 and ZBTB16. Further the following fusions were found: NPM1-RP3P0, NPM1-SETBP1, NUP98-ING3, IRF2BP1-RARA, and ZBTB16-RARA. Thus, RNA sequencing identified 39 fusion transcripts, which standard diagnostics had not identified as one of the partner genes. Failure to detect gene fusions should initiate improvements in calling algorithms and may also have biological implications. It was reported that genomic rearrangements of RUNX1 occur, which do not lead to RUNX1 in frame fusion transcripts but to termination of transcription.

Summary/Conclusions: 1) RNA sequencing was able to detect recurrent gene fusions with high accuracy and to characterize rare gene fusions providing the basis for the design of RT-PCR based assays for monitoring MRD. 2) Targeted RNA sequencing may be a valuable tool in routine diagnostics for patients with rearrangements unresolved by standard techniques. 3) These findings may have consequences for targeted treatment approaches.

Background: Mixed phenotype acute leukemia (MPAL) is a rare subgroup of acute leukemia characterized by blasts that show immunophenotypes of both myeloid and lymphoid lineages and therefore not traceable to single lineage of origin. Diagnosis of MPAL is challenging due to the possible discrepancy between immunophenotype and morphology. Clinically, MPAL has poor prognosis and poses therapeutic challenges. Genetic basis of MPAL is not well understood.

Aims: To clarify the underlying pathogenesis of MPAL and provide clue on future personalized therapy in MPAL, we performed comprehensive molecular characterization of adult MPAL.

Methods: We studied 31 adult patients with MPAL (median age 53) that met 2008 AML criteria. For the initial treatment we showed that 14/25 (56%) patients were studied by targeted capture exome sequencing of 295 genes that are recurrently mutated in hematologic malignancies (median 393x coverage, N=31), RNA sequencing (N=24), and Infinium methylation EPIC array (Illumina, N=31). Mutational landscape was compared to that of 194 AML, 71 B-ALL, and 6 T-ALL (40 cases of which pre-treatment samples were sequenced internally with the same platform. Promoter CpG methylation pattern was compared to the data from 194 AML (data derived from The Cancer Genome Atlas project), 505 B-ALL and 101 T-ALL cases (data shared by Nordlund et al. Genome Biology. 2013). Copy number variation was inferred from methylation array data.

Results: Among 31 MPAL cases, 15 (48%) had myeloid-T and 13 (42%) had myeloid-B phenotype. Four cases had Philadelphia chromosome, 1 had 11q23 abnormality, and 8 had complex karyotype. MPAL had similar numbers of mutations (median 2 [range: 0-8]) with AML (median 3 [range: 0-7], P=0.79) or T-ALL (median 3 [range: 1-4]. P=0.92) but had significantly higher number of promoter CpG methylation (median 0 [range: 0-4], P=0.04) with higher similarity to the mixed immunophenotypic features, MPAL had both AML-type and ALL-type mutations. However, NPM1 mutation was specific to AML and was not found in MPAL cases. Myeloid-T and myeloid-B showed distinct patterns of somatic mutations. Genes in which mutations were enriched in myeloid-T than in myeloid-B included DMNT3A (33% vs 8%), IDH2 (33% vs 8%), NOTCH1 (41% vs 0%), IL7R (17% vs 0%), and FBXW7 (6% vs 0%). Genes in which mutations were less frequently observed in myeloid-T than in myeloid-B included RUNX1 (6% vs 46%), ASXL1 (0% vs 23%), TET2 (0% vs 15%), SRSF2 (6% vs 23%), and FLT3 (11% vs 23%). Myeloid-T and myeloid-B showed distinct patterns of promoter CpG methylation. Over all, we confirmed that the CpG loci than myeloid-B in all different CpG locations (island, shore, shelf, and others). Genes that are essential in T-cell receptor (TCR) signaling (CD3D, CD7, CD247, LCK, PRKRCO, ccr9, and TCL1A) were differentially methylated and consequently differentially expressed between myeloid-T and myeloid-B. Copy number variation analysis showed that 80% of the cases harbored one of the essential NOTCH pathway key genes, was amplified and was overexpressed. RNA sequencing revealed several known translocations such as NUP98-NSD1 and KMT2A-MLLT4, in addition to the novel translocations such as FOX2P1-DNAJC15, FLI1-ITFT46, and ITFPR2-ARID8S. Unsupervised hierarchical clustering of all MPAL, AML, B-ALL and T-ALL by promoter CpG methylation pattern revealed that myeloid-T consistently showed similar methylation pattern with T-ALL, while myeloid-B showed random similarity with either B-ALL or AML.

Summary/Conclusions: MPAL is genetically heterogeneous disease and myeloid-T and myeloid-B shows distinct patterns of mutation landscapes, promoter CpG methylation and gene expression. Therapy for MPAL may need to be personized based on genomic profiles.

P549

FEASIBILITY AND BENEFIT OF TARGETED RNA SEQUENCING FOR THE DETECTION OF RECURRENT FUSION TRANSCRIPTS AND THE IDENTIFICATION OF NOVEL FUSION TRANSCRIPTS IN MYELOID MALIGNANCIES

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P550

COMPREHENSIVE MOLECULAR ANALYSIS OF ADULT MIXED PHENOTYPE ACUTE LEUKEMIA (MPAL)

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P551

THE EFFECTS OF EARLY INTENSIFIED INDUCTION CHEMOTHERAPY IN ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA COMPARED TO STANDARD ANTHRACYCLINE PLUS CYTARABINE 3+7 CHEMOTHERAPY D.-H. Kwak1,2,*, H.-J. Yoon1, H.-J. Kim1, S.-S. Park1, S.-E. Lee1, B.-S. Cho1, K.-S. Eom1, Y.-J. Kim1, S. Lee1, J.-W. Lee1, W.-S. Min1
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haematologica | 2017; 102(s2) | 211

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men (standard group). Re-induction and consolidation therapy was performed according to a consistent strategy and post-remission therapy was mainly based on hematopoietic cell transplantation.

Results: Early intensified group was consisted of younger patients (median age, 37 years old [range 17-69]) vs 45 years in 3+7 vs 43 years in 3+10 subgroup) and larger proportion of b(2;11) (n=102 [27.7%] vs 73 [3.3%] vs 3+10 subgroup, 12.9%), P=0.001. Also, initial GM blast counts were higher in two intensified groups (73.3% in 5+10 and 70.1% in 3+10) compared to 3+7 subgroup (66.8%, P<0.001). Early death rate at 8 weeks was higher in patients older than 55 years (10.8% vs 3.7%, P<0.001) especially when they were treated with intensified chemotherapy (21.7% vs 15.7% in 3+10 vs 6.3% in 5+10, P<0.038). CR rate after induction was high in young patients especially in 3+10 subgroup (79.8%, P=0.001) and we also found that patients with favorable to intermediate-risk karyotype might benefit with intensified chemotherapy in the context of CR rate (79.7% vs 68.3%, P<0.001, although final CR rates became similar after re-induction. Next, we found that pre-HCT relapse rate was lower in patients younger than 55 years (4% vs 0%, P=0.002) and favorable to intermediate-risk group (8.9% vs 20.2%, P<0.001) after intensified induction. In young patients with favorable to intermediate-risk karyotype, intensified group showed superior 5-year OS (55.0% vs 45.5%, P=0.010) and lower long-term relapse rate (32.2% vs 38.0%, P=0.084), but multivariate analysis revealed no effects for both OS and CR. In patients older than 55 years, intensified group showed inferior 5-year OS (19.2% vs 22.8%, P=0.014) with higher early death rate (17.6% vs 6.3%, P=0.015), and multivariate analysis also showed intensified induction was related inferior OS (HR=1.89, 95%CI; 1.14-3.15, P=0.013).

Summary/Conclusions: Our data revealed that intensified induction chemotherapy was not influential for poor-risk karyotype, while higher post-induction CR rate and low pre-HCT relapse was shown in young patients with favorable to intermediate-risk karyotype although it was not influential for final OS and CR rate. In elderly patients, intensified induction chemotherapy was related with higher early death rate which finally showed poor OS.

P552

VARIANT FLT3 MUTATIONS CAN BE ERADICATED BY CYTARABINE/ANTHRACYCLINE/CRENOLANIB INDUCTION IN ADULT PATIENTS WITH NEWLY DIAGNOSED FLT3 (ITD/TKD) MUTANT AML

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Background: Patients (pts) with FLT3-internal tandem duplication (ITD) and FLT3-D835 mutant AML have a high relapse rate. These relapses are typically due to outgrowth of mutant FLT3 clones. Previously available PCR-based tests only checked for presence of FLT3-ITD and FLT3-D835/B836 mutations. Whole genome sequencing of 799 pediatric AML samples from COG trials have shown novel FLT3 variants in mainly the tyrosine kinase domain also in juxtamembrane (JM) and transmembrane domains in 7.6% of these samples (Tarlock et al. ASH 2015). Some of these mutations result in autophosphorylation of FLT3 and therefore may be oncogenic.

Methods: Pts with newly diagnosed FLT3 mutant AML were enrolled and treated with cytarabine/anthracycline/crenolanib induction followed by high dose cytarabine (HiDAC) consolidation. Crenolanib 100mg TID was started on day 9 of induction chemotherapy. Crenolanib was then continued till the next chemotherapy. Following consolidation and allogeneic stem cell transplantation. Bone marrow samples were collected at baseline and at the time of remission assessment. Sequencing of the entire FLT3 gene was performed through FoundationOne Heme panel (n=18) and MSKCC multigene panel (n=5). Sequencing of exons 14,15,16, and 20 was performed through the Rapid Heme Panel at Dana-Farber Cancer Institute in additional 6 pts.

Results: Out of 29 newly diagnosed FLT3 mutant AML patients with full/partial FLT3 gene sequencing performed, 4 pts were found to have novel variant FLT3 mutations consisting of V491L, V592L, D593H, A686V, and N841IT (Table 1). The majority of these novel mutations were located at the JM, kinase domain 1 and the activation loop (kinase domain 2). The whole allele fractions of these FLT3 variants ranged as high as 29% (higher than that of FLT3-ITD in p3), suggesting that some of these clones may have been potentially driving clinical leukemia progression in some pts. All 4 pts had NPM1 mutations, and two also had DNMT3A mutations. All 4 pts achieved CR with full count recovery (3/4 pts achieved CR after just one cycle of cytarabine/anthracycline/crenolanib induction). The pt with FLT3-D835Yand N841T achieved a CR after cytarabine/anthracycline/crenolanib induction and one cycle of HiDAC consolidation. All pts treated with FLT3-ve and have received FLT3-ve out of 4 pts received 1-4 cycles of HiDAC consolidation followed by crenolanib maintenance. Only one pt underwent allo SCT. With a median follow up of 13 months, one pt relapsed (at 8.4-month following treatment). This 61F pt was found to have FLT3-ITD, D593H and i836del FLT3 abnormalities at the time of diagnosis. A full FoundationOne gene panel done at the time of relapse, showed no residual FLT3 mutant clones.

Summary/Conclusions: This abstract reports multiple novel variant FLT3 mutations in adult pts with newly diagnosed FLT3-ITD or FLT3-D835 mutant AML. The allelic burden of these FLT3 variant mutations can sometime be higher than that of FLT3-ITD. Detailed FLT3 analyses in this subset of pts suggests that crenolanib in combination with standard induction chemotherapy has the ability to eradicate variant FLT3 clones. All 4 pts treated with chemother-apy followed by crenolanib showed clearance of FLT3-ITD, TKD, as well as other novel variants. To achieve maximal clinical benefit, a potent pan-FLT3 inhibitor with the ability to inhibit IDT, DB35, as well as other activating mutations maybe beneficial.

P553

PATIENTS WITH ACUTE MYELOID LEUKEMIA WHO HAVE MUTATIONS IN IDH1 OR IDH2 RESPOND WELL TO INDUCTION CHEMOTHERAPY WITH "7+3" DESPITE THE PRESENCE OF COMPLEX KARYOTYPE OR FLT3-ITD

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Background: Mutations in isocitrate dehydrogenase isoforms 1 and 2 (IDH1/IDH2) occur in 8-12% of patients with acute myeloid leukemia (AML). Mutant IDH enzymes catalyze the conversion of alpha ketoglutarate to beta hydroxyglutarate. Increased concentrations of intracellular 2-HG lead to histone hypermethylation and a block in cellular differentiation and may also lead to suppression of homologous recombination. Previous studies of outcomes in patients with FLT3-ITD have shown that the addition of a hypomethylating agent to 7+3 induction regimens is beneficial. In this study, we investigated the outcomes of patients given induction chemotherapy with daunorubicin and cytarabine (7+3), the most common regimen used in the United States.
Aims: To delineate the complete remission rate in AML patients with IDH1 or IDH2 mutations who receive standard 7+3 induction chemotherapy.

Methods: After receipt of IRB approval, an institutional database of genomic abnormalities in all patients with AML was queried for patients with IDH1 or IDH2 mutations between the years of 2010 and 2016. Pathology records of patients identified as having an IDH1/IDH2 mutation were reviewed to confirm the presence of an IDH mutation. After confirmation of IDH mutational status, all patients who received standard induction chemotherapy with 7+3 were included in this retrospective chart review.

Results: Between 2010 and 2016, 82 patients with IDH1/IDH2 mutations who had been treated with 7+3 induction chemotherapy were seen at MSKCC. Of these, 33 (40.2%) had IDH1 mutations and 49 (59.8%) had IDH2 mutations. Of those with IDH2 mutations IDH2 R140Q mutations were present in 34 (69.3%) and IDH2 R172K mutations were present in 15 (30.6%). The median age of all patients treated was 63. 56 patients (68%) had de novo AML, 16 (20%) had AML with myelodysplasia related changes, 5 (6%) had a known prior history of MDS and 5 (6%) had therapy related AML. Nearly half of the patients (49%) had karyotypic abnormalities. Of the 82 patients who received induction chemotherapy with 7+3, 51 achieved a complete remission (CR) after 1 cycle and 16 after 2 cycles for a CR rate of 82%. The strongest predictor of response to induction chemotherapy was the presence of an NPM1 mutation. There was a trend towards decreased response to induction chemotherapy in patients with a complex karyotype (p=0.079) that did not reach statistical significance.

The presence of an IDH2 R172K mutation was predictive of non-response to one cycle of (7+3) of 7+3 but when two cycles of induction chemotherapy were given, response rates were equivalent to patients with R140Q mutations. Co-occurring mutations in FLT3 (ITD or TKD), DNMT3A or NRAS were not predictive of responses to induction chemotherapy.

Summary/Conclusions: Induction chemotherapy with 7+3 leads to a robust CR rate of 82% in patients with AML that harbor and IDH1 or IDH2 mutation. CR rate is not affected by FLT3 mutations, although patients with IDH2 R172 mutations required two cycles of chemotherapy to achieve a remission. Karyotypic abnormalities did not influence the response to induction chemotherapy, nor did the presence of co-occurring FLT3-ITD, FLT3-TKD or RAS mutations require two cycles of chemotherapy to achieve a CR.

Background: Treatment of Acute Myeloid Leukemia (AML) is limited to few different treatments in each clinical trial group guideline, but integrating current and previous guidelines, and clinical trial publications, there are up to 45 drug combination treatments among approved chemotherapy drugs in Europe and USA. There is a need for Precision Medicine (PM) tests to identify which of these different treatments maybe optimal for each individual patient, independently of where he/she lives.

Aims: To provide actionable data to improve disease management with existing treatments with a PM test to guide the hematologist among all possible treatments to achieve a CR.

Methods: AML bone marrow (BM) samples from adult patients were received at the laboratory within 24 hours from extraction and incubated for 48h in 96-well plates containing single drugs or combinations representing up to 45 different treatments that are currently given in the clinical practice. The analysis is performed in the automated flow cytometry PharmaFlow platform, 72 hours after the extraction of the sample, an encrypted report is sent to the hematologist before the patient begins treatment. Pharmacological responses were calculated using pharmacokinetic population models. Induction response was assessed according to the Cheson criteria (2003). Patients attaining a CR/CRi were classified as responders and the remaining as resistant, excluding early deaths. Final scores and treatments ranking is based on a therapeutic algorithm that integrates ex vivo activity; monotherapy dose responses quantified by the area under the curve (AUC) with limits such as Cmax values, and synergism calculated measuring 8 concentration ratios requiring consistency in their results in a 3D surface (so called alpha factor synergism). The PM Test attempts to identify at least one treatment, among all evaluated alternatives, predicted sensitive for each patient; conversely, if sensitive treatments can be identified the PM Test can provide the hematologist with valuable guidelines for individualized treatment.

Results: (Figure 1) The scoring method was tested using ex vivo results from samples obtained in an observational clinical trial with Spain’s PETHEMA group from a cohort of 123 samples from de novo diagnosed AML patients, treated with the standard PETHEMA 1st line guideline 3+7 with CYT+IDA. The score predicts sensitive patients with 90% accuracy. This accuracy can be compared with an independently derived 92% accuracy in identifying sensitive patients in a statistically significant clinical correlation study (EHA Poster 2016 Montesinos et al.). The score is a simplified version of such correlation algorithm. Both methods identify a similar % of all clinically sensitive patients (67% vs 71%).

Acute myeloid leukemia - Clinical 5
However, the correlation is only valid for CYT-IDA while the PM Test is applied to up to 45 treatments. Any such treatment identified as sensitive means the PM Test can provide a valuable guideline to hematologists. This means the PM Test can suggest sensitive treatments for the vast majority of patients.

Figure 1.

Summary/Conclusions: We have developed a novel ex vivo PM test for induction treatment in AML patients to guide hematologists selecting the right treatment to achieve CR in individual patients levering up to 45 different validated chemotherapeutic regimes. Assuming a similar response rate for all these treatments, our test could estimate a net prediction for sensibiity to AML treatment higher than 80% in 1st line. This PM Test will be evaluated in an interventional clinical trial on relapse/refractory patients that is expected to begin in the next few months in collaboration with the PETHEMA group from Spain.

P555

RESPONSE-ADAPTED AZACITIDINE AND INDUCTION CHEMOTHERAPY IN PATIENTS ≥60 YEARS OLD WITH NEWLY DIAGNOSED AML ELIGIBLE FOR CHEMOTHERAPY: RESULTS OF THE DRKS00004519 STUDY OF THE EAST GERMAN STUDY GROUP

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Background: AML treatment in elderly patients (pts) ≥60 years (y) with intensive chemotherapy (IC) or azacitidine (AZA) are not necessarily mutually exclusive. Aims: Results of the multicenter DRKS00004519 (RAS-AZIC) study of the East German Study Group (OSHO) which evaluated first-line treatment with AZA following by response-based AZA or IC in pts >60y with AML are presented.

Methods: pts >60y with newly diagnosed AML (n=112) were included. Recruitment was completed in May, 2016. In the phase I part, safety of upfront AZA (75mg/m2/day s.c) for 7 days followed by IC (mitoxantrone 10mg/m2/day on day (d) 1-3 and cytarabine 1g/m2/BID on d 1, 3, 5, 7) on d17 was established through a 3+3 design. In the multicenter phase II part (figure), upfront AZA was sequentially followed by AZA or IC based on d15 bone marrow (BM) blasts (<45 vs >45%) and CR/CRi on d56 which were both previously identified as early predictors for long-term response to AZA in AML (Ai-Al et al. Leuk Lymph 2016). Treatment failure was defined as disease progression (DP) or death. ORR was evaluated according to the International Working Group criteria. Based on the optimal two-stage design (Simon. Control Clin Trials 1989), protocol treatment was non-inferior to standard IC if, on an intention-to-treat basis, an OR of 61% was reached. Adverse events (AEs) were reported according to the NCI CTCAE 4.03. All pts gave written informed consent.

Results: Median age was 70y (52% males), de novo AML was present in 65% of pts. Median BM blasts and WBC were 50% and 4.4x109/L respectively. Genetic risk was high in 30%, intermediate in 55%, and favorable in 15%. FLT3 and NPM1 were mutated in 12% and 22% respectively. All pts received first-line AZA. Only lower baseline blasts correlated with blast <45% on d15 (p=0.0005). Yet, 40% of pts with baseline blasts >50% reached this goal. Protocol assigned treatment on d15 was applied to 101 (90.2%) pts (54.5% continued with AZA; 46.5% received IC). Of 152 AZA cycles given till d56, 33.6% were applied in an outpatient setting. Until d90, one IC cycle was needed in 77 (68.8%) pts. In the intention-to-treat cohort (n=43%/15%); PR (4.5%) and CR/CRi were 62.5% [CR/CRi (n=43%); PR (4.5%)] and 8.9% respectively. The probabilities of achieving CR/CRi with AZA alone, two AZA cycles + one IC, and one AZA cycle + one IC were 28.3%; 53.3%; and 58.3% respectively. Age, WBC, and type of AML had no impact on response in the three treatment scenarios. Similarly, response was not influenced by baseline BM blasts. CR/CRi was lower in high risk genetics (48%) compared to other risk categories (78%) (p=0.007). This negative association was particularly marked in pts with high-risk genetics and d15 BM blasts >45% [CR/CRi 38.5% vs 84% in other genetic categories (p=0.009)]. Interestingly, the impact of genetics on OR was not seen in the two AZA cycles + one IC cohort (p=1.0). CR with AZA alone was remarkably high (70%) in pts with favorable genetics including those with NPM1mut/FLT3wt (p=0.003). Protocol therapy was generally well tolerated. Constipation grade 1+2 was the most frequently reported AE under AZA (48%). The most frequent grade 3+4 non-hematologic AE was infection (IC [47%]; AZA [20%]).

Figure 1.

Summary/Conclusions: We have developed a novel ex vivo PM test for induction treatment in AML patients to guide hematologists selecting the right treatment to achieve CR in individual patients leveraging up to 45 different validated chemotherapeutic regimes. Assuming a similar response rate for all these treatments, our test could estimate a net prediction for sensibility to AML treatment higher than 80% in 1st line. This PM Test will be evaluated in an interventional clinical trial on relapse/refractory patients that is expected to begin in the next few months in collaboration with the PETHEMA group from Spain.

P556

OVERALL SURVIVAL WITH CPX-351 VERSUS 7+3 IN OLDER ADULTS WITH NEWLY DIAGNOSED, THERAPY-RELATED ACUTE MYELOID LEUKEMIA: SUBGROUP ANALYSIS OF A PHASE 3 STUDY

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Background: Therapy-related acute myeloid leukemia (tAML) may occur as
a late complication of cytotoxic or radiation therapy and is associated with a poor prognosis. CPX-351 is a liposomal formulation that delivers a synergistic 5:1 molar ratio of cytarabine and daunorubicin. In a randomized, open-label, controlled phase 3 trial in patients aged 60 to 75 years with newly diagnosed, secondary AML (eg, tAML or AML after myelodysplastic syndrome), CPX-351 significantly improved overall survival (OS) versus cytarabine/daunorubicin (7+3).

Aims: The current analysis of this phase 3 study evaluated outcomes in the subgroup of patients with tAML.

Methods: Enrolled patients were randomized 1:1 to receive induction with 1 to 2 cycles of CPX-351 (100 units/m² [cytarabine 100mg/m² + daunorubicin 44mg/m²] on Days 1, 3, and 5 [2nd induction: Days 1 and 3 only]) or 7+3 (cytarabine 100mg/m²/day x 7 days [2nd induction: x 5 days] + daunorubicin 60mg/m² on Days 1, 2, and 3 [2nd induction: Days 1 and 2 only]). Patients who achieved complete remission (CR) or CR with incomplete platelet or neutrophil recovery (CRi) could receive up to 2 cycles of consolidation therapy. Note, the study was not powered for this subgroup analysis.

Results: A total of 304 patients were enrolled and received study treatment, including 62 (20%) patients with tAML (CPX-351 arm, n=30; 7+3 arm, n=32). Characteristics of tAML patients were similar between the CPX-351 and 7+3 arms: median age was 69.0 versus 67.5 years, and 47% versus 53% were male. Prior treatment in patients with tAML included prior non-anthracycline chemotherapy alone (26%), radiation alone (26%), non-anthracycline chemotherapy + radiation (32%), non-anthracycline + anthracycline chemotherapy (5%), and non-anthracycline + anthracycline chemotherapy + radiation (11%). CPX-351 was associated with a significant OS benefit versus 7+3 in older tAML patients and numerically longer event-free survival and remission duration (Figure). Additionally, a greater proportion of tAML patients in the CPX-351 arm versus the 7+3 arm achieved CR+CRi (47% vs 38%, respectively; odds ratio=1.33 [95% CI: 0.47, 3.81]) and proceeded to stem cell transplantation (37% vs 27%; odds ratio=1.54 [95% CI: 0.53, 4.49]). Serious treatment-emergent adverse events (TEAEs) were reported for 18/30 (60%) of tAML patients in the CPX-351 arm and 12/32 (38%) of tAML patients in the 7+3 arm; the observed difference in serious TEAEs in this subpopulation appeared to primarily be due to the incidence of febrile neutropenia (n=6/30 [20%] vs n=0/30 [0%]). Treated patients in the 7+3 arm experienced a TEAE that resulted in death during the treatment period; there was no pattern in the individual TEAEs that led to death.

Figure 1.

Summary/Conclusions: CPX-351 is associated with improved efficacy and a safety profile comparable to 7+3 in older patients with newly diagnosed tAML. Outcomes in the tAML subgroup mirrored the overall study population, indicating CPX-351 may represent a new therapeutic option for this difficult to treat population.

P557

HYPERFERRITINEMIA IS AN INDEPENDENT POOR PROGNOSTIC FACTOR IN ACUTE MYELOID LEUKEMIA

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Background: The prognostic impact of ferritinemia has been studied in myelodysplastic syndromes and acute myeloid leukemia (AML) patients undergoing allogeneic stem cell transplantation (SCT). In this context, high levels of serum ferritin have been correlated to a shorter overall survival (OS) and an increased relapse risk. We have previously shown that hyperferritinemia at diagnosis has a strong prognostic impact in a cohort of 162 AML patients with intermediate cytogenetic risk and younger than 60.

Aims: We now extend the analysis to all age and cytogenetic risk, in order to confirm the impact of hyperferritinemia in AML.

Methods: This study included 525 adult AML patients (excluding acute promyelo-cytic leukemia) treated by intensive chemotherapy in Toulouse and Lyon University Hospitals between January 1st, 2005 and December 31st, 2014 who had ferritinemia documented at AML diagnosis. Ferritin level was measured by specific spectrophotometry. Primary outcome was disease-free survival (DFS). To avoid the loss of information and the reduction in power introduced by the categorization of ferritinemia and to deal with the non-linearity in the relationship between outcomes and ferritinemia, we explored the relationship between ferritinemia and outcomes using restricted cubic spline.

Results: Median age at diagnosis was 59.4 years (interquartile range [IQR], 47.8-66.4); 303 of them (57.7%) were men. Disease status was de novo in 83.2% (N=437). Median white blood cell count (WBC) was 10.0x10⁹/L (IQR, 2.5-41.5). Cytogenetic risk was favorable, intermediate and adverse in 9.2% (N=48), 71.8% (N=374) and 19% (N=99) respectively; ELN classification was favorable, intermediate-I, intermediate-II, adverse and unknown in 21.0% (N=110), 25.5% (N=134), 22.3% (N=117), 18.9% (N=99) and 12.4% (N=65) respectively. Median ferritinemia at AML diagnosis was 715 µg/L (IQR, 372-1304), ranging from 34µg/L to 70759 µg/L (upper normal limit [UNL], 300µg/L). 421 patients achieved complete remission (CR); 80.2%. Early death and treatment failure rates were 7.8% (N=41) and 12% (N=63) respectively. 169 patients underwent allogeneic HSCT in first CR (32.2%). Median DFS was 19.8 months (IQR, 8.4-Not Reached). Ferritinemia had a significant impact on DFS: median DFS was 21.2 months in patients with ferritinemia ≤2100 µg/L (7-fold UNL), and 12.7 months with ferritinemia >2100 µg/L (HR, 1.6 [95%CI, 1.1-2.3], p=0.0253). After adjustment for age, AML status and cytogenetics or ELN classification, relapse or death rate significantly (p=0.0122) increased from ferritinemia superior or equal to 2141 µg/L (Figure 1). Ferritinemia had also a significant impact on early deaths, CR rate, EFS and OS after adjustment (≥4-fold UNL, p<0.0001; ≥3-fold UNL, p=0.004; ≥2-fold UNL, p<0.0001 and ≥3-fold UNL, p=0.01 respectively).

Figure 1.

Summary/Conclusions: In conclusion, hyperferritinemia is a prognostic marker independent from well-acknowledged factors, such as cytogenetics and molecular abnormalities. Ferritinemia should be included at AML diagnosis workup as it provides reproducible information on short and long-term outcome for AML patients of any subgroup. The putative link between hyperferritinemia, inflammation and chemoresistance should be investigated.

P558

NGS ANALYSIS OF 474 BONE MARROW SAMPLES FROM 157 AML PATIENTS TREATED WITH AZACITIDINE—IMPACT OF AGE ON MUTATIONAL LOAD

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Background: Recent publications have shown the prognostic value of paired molecular analyses in patients (pts) with acute myeloid leukaemia (AML) (Papaemmanuil et al, NEJM 2016). While recent data has been published on pts with myelodysplastic syndromes (MDS) and AML treated with decitabine, (Welch et al, NEJM 2016; Duncavage et al, Blood 2017) data on
AML pts treated with azacitidine (AZA) has only been presented in abstract form thus far (Tang et al, ASH 2016). Data on the impact of age on mutational load in AML are scarce.

Aims: To assess the mutational landscape in elderly AML pts treated with AZA; specifically, whether age has an impact on mutational load.

Methods: We analysed 474 bone marrow FFPE specimens from 157 AML pts in the Austrian Registry of Hypomethylating Agents from two centers (Salzburg, Wels-Grieskirchen) using a 53-gene panel (all exons). NGS was performed by Qiagen. Minimum coverage: 1.500x. All mutations were checked against COSMIC v79, ClinVar, ICGC, DoCM, dbSNP and Varsome databases. For comparison of categorical variables Chi-squared test was used, for comparison of means Students’ T-test was used.

Results: The rate of secondary (s)AML was significantly lower in pts <75 (n=85), vs ≥75 years (n=54) (66.0 ± 77.8%, p<0.001). There was no significant difference in the rate of adverse cytogenetics or monosomal karyotype before AZA treatment between pts < vs ≥75 years, respectively (data not shown). Mutational load (average number of mutated genes and mutations per pt) assessed at/before initiation of AZA, was significantly higher in pts <75 vs pts ≥75 years (10.2 ± 8.6 mutated genes/pt; p=0.020 and 12.9 ± 10.5 mutations/pt; p=0.012; Figure 1A). This also held true when mutational load was assessed at any timepoint during the course of AML including during/post-AZA treatment (Figure 1B). In total, 139 pts had more than one marrow sample with NGS results. Analysis of paired samples revealed that mutational load was significantly higher during/post-AZA vs before AZA in both age groups (Figure 1C-D). In total, 60.4%, 15.8%, 8.6%, 3.6% and 11.5% of pts acquired 0, 1, 2, 3, 4-13 additional mutations, respectively. No relevant differences between pts < vs ≥75 years were found (data not shown). When comparing the delta of mutations before vs during/after AZA according to age group, no significant difference was found (Figure 1E).

Table 1.

<table>
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<th>Group</th>
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<td>n pts. in %</td>
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<tr>
<td>Mutated genes in %</td>
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<tr>
<td>Mutated genes per pt.</td>
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<td>Mutations per pt.</td>
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<tr>
<td>Mean number of mutations per pt</td>
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<td>12.9 ± 10.5</td>
<td>0.012</td>
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</table>

Summary/Conclusions: The observed mutational load per pt in our cohort is higher than that observed by others using targeted re-sequencing methods, which report an average of only 2-4 mutations per pt (Duncavage et al, Blood 2017; Conte et al, Leuk 2013; Au et al, Diagn Pathol 2016; Grove & Vassiliou, Dis Model Mech 2014). It seems however, that a higher mutational load (average number of mutated genes and mutations per pt) assessed at/before initiation of AZA, was significantly higher in pts <75 vs ≥75 years (10.2 ± 8.6 mutated genes/pt; p=0.020 and 12.9 ± 10.5 mutations/pt; p=0.012; Figure 1A). This also held true when mutational load was assessed at any timepoint during the course of AML including during/post-AZA treatment (Figure 1B). In total, 60.4%, 15.8%, 8.6%, 3.6% and 11.5% of pts acquired 0, 1, 2, 3, 4-13 additional mutations, respectively. No relevant differences between pts < vs ≥75 years were found (data not shown). When comparing the delta of mutations before vs during/after AZA according to age group, no significant difference was found (Figure 1E).

P559

PROGNOSTIC VALUE OF EARLY WT1 RESPONSE IN AML PATIENTS UNDERGOING INTENSIVE CHEMOTHERAPY

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Background: Monitoring minimal residual disease based on quantitative PCR represents an important risk stratification tool in acute myeloid leukemia (AML) and enables the prediction of impending relapse. Besides common fusion genes and mutated genes, Wilms tumor 1 (WT1) gene is widely used to follow de novo AML.

Aims: The aim of our study was to evaluate the relevance of WT1 expression for the prognosis of patients with AML in a real life population.

Methods: We analysed morphological samples from 174 consecutive adult AML patients (18-85 years) who treated with AZA and performed WT1 detection for all AML patients. AML patients were excluded. Of 143 patients with WT1 overexpression at diagnosis, those treated with intensive induction chemotherapy and achieving haematological remission after the first cycle of therapy were included in the retrospective follow-up analysis (n=129).

Results: The extent of WT1 expression at diagnosis had no prognostic relevance. In contrast, achievement of low WT1 levels after induction chemotherapy was associated with a significant better overall (OS) and disease free survival (DFS) as compared to persistent high WT1 expression at hCR1 5 years OS 0% vs 77.8% (p<0.001); DFS 44% vs 0% (p<0.001). Additionally, compared to patients with a low WT1 reduction (<5 log) at hCR1, the relative risk of death was 0.32 (95% CI 0.1-0.7) in patients with intermediate WT1 reduction (5-8 log) and 0.15 (95% CI 0.0-0.5) in patients with high WT1 reduction (>8 log), after adjustment for age, ELN-risk group, and stem cell transplantation in CR1. The corresponding results in patients with low, intermediate and high WT1 reduction were 10%, 42% and 71% (p<0.001), respectively. Even though numbers of patients were small (n=33), SCT at CR1 seems to overcome the adverse risk of persistent WT1 expression: DFS 5.3 years (0-12.9) for patients with SCT and 0.7 years (0-6.0) for patients without SCT (p=0.004).

Summary/Conclusions: Persistent WT1 expression in AML patients achieving a CR1 after induction chemotherapy is a strong, independent predictor for DFS and OS in patients with AML. Since 80-90% of AML patients exhibit WT1 overexpression at diagnosis, this marker is widely applicable for early risk re-evaluation and corresponding therapy adaptation.

P560

EVALUATION OF THE IMPACT OF SIGNAL RATIO ON OVERALL SURVIVAL IN FLT3-MUTATION-POSITIVE RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA FOLLOWING ONCE-DAILY TREATMENT WITH GILTERITINIB


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Background: Fms-like tyrosine kinase 3 (FLT3) internal tandem duplications (ITD) in acute myeloid leukemia (AML) are associated with early relapse and short survival, particularly in the context of high allelic burden. Patients with high FLT3-ITD signal ratio are particularly sensitive to FLT3 inhibitors but the clinical effects of allelic burden on survival have not been validated in trials of these drugs. Gilteritinib is a highly specific, potent FLT3/AXL inhibitor with demonstrated activity against both FLT3-ITD and tyrosine kinase domain (TKD) mutations. A recent Phase 1/2 study (CHRYSALIS; NCT02014558) demonstrated that FLT3 mutation-positive (FLT3mut+) patients with relapsed/refractory (R/R) AML treated with gilteritinib had high clinical response rates and prolonged overall survival (OS), especially at doses ≥80mg/d.

Aims: To evaluate the effect of FLT3-ITD and FLT3-TKD signal ratios on OS and survival in FLT3mut+R/R AML patients who had received gilteritinib doses ≥80mg/d.

Methods: Signal ratios were assessed in adult FLT3mut+R/R AML patients who had received gilteritinib doses ≥80mg/d. Genomic DNA extraction and PCR with fluorescent primers were used to generate transcripts of FLT3 alleles containing ITD and TKD mutations. A recent Phase 1/2 study (CHRYSALIS; NCT02014558) demonstrated that FLT3 mutation-positive (FLT3mut+) patients with relapsed/refractory (R/R) AML treated with gilteritinib had high clinical response rates and prolonged overall survival (OS), especially at doses ≥80mg/d.

Aims: To evaluate the effect of FLT3-ITD and FLT3-TKD signal ratios on OS and survival in FLT3mut+R/R AML patients who had received gilteritinib doses ≥80mg/d.
present in the wild-type allele. Differential fluorescence detection enabled identification of the specific transcripts. Median ITD signal ratio (ie, FLT3-ITD/wild-type FLT3) was calculated from patients with ITD mutations (without concomitant TKD mutations); median TKD signal ratio (ie, FLT3-TKD/wild-type FLT3) was calculated from all patients with a TKD mutation. Median OS estimates were derived and stratified based on ITD and TKD signal ratios that fell above or below median signal ratio values reported for the trial.

Results: Signal ratio was assessed in 152 patients with FLT3-ITD and -TKD mutations who had received ≥80mg gilteritinib. Of these patients, 136 had FLT3-ITD mutations with or without concomitant TKD mutations, and 16 had FLT3-TKD mutations only. Median ITD and TKD signal ratios were 0.84 and 0.5, respectively. Patients with FLT3-ITD signal ratios that were above or below the median ITD signal ratio had OS durations of 216 and 213 days, respectively. No significant difference in median OS was observed between patients in the highest and lowest FLT3-ITD signal ratio quartiles (Figure 1). Patients with TKD signal ratios that were above the median value (≥0.5) had a median OS of 202 days; those with TKD signal ratios below the median value had a significantly shorter median OS of 33.5 days (P<0.0004; Figure 1).

Figure 1.

Summary/Conclusions: These data show that FLT3-ITD signal ratio has little impact on survival in patients with FLT3-ITD mutations who received gilteritinib. In the small number of patients with FLT3-TKD mutations only, high TKD signal ratio was associated with a longer OS, similar to that observed in patients with FLT3-ITD mutations. These data suggest a possibility that oncogene addiction in FLT3-TKD+ R/R AML requires a high allelic burden and clonal dominance. Also, it is possible that FLT3-ITD signal ratio in R/R AML may contribute to the response rate in patients with FLT3-TKD mutations only. Further investigation is warranted.

P561

CLINICAL OUTCOME OF HYPOCELLULAR AML AND AML WITH MYELODYSPLASIA-RELATED CHANGE (MRC) COMPARED TO DE NOVO ADULT AML WITH NORMAL CELLULARITY AFTER HEMATOPOIETIC CELL TRANSPLANTATION


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Background: Hypocellular acute myeloid leukemia (hypo-AML) and AML with myelodysplasia-related change (AML-MRC) accounts for small proportion of adult AML. As the characteristics and outcomes are not well recognized.

Aims: We tried to analyze these specific groups and compared to normocellular AML.

Methods: After exclusion of secondary AML, therapy-related AML, and AML M3, we retrospectively analyzed 1593 AML cases between 2002 and 2013. We found 101 (6.3%) patients with hypo-AML and 164 (10.3%) patients with de novo AML/MRC. Hypo-AML was diagnosed with blast counts ≥20% within hypocellular (<20%) bone marrow (BM) or hypocellular BM with ≥10% multilineage dysplasia. AML-MRC was defined with multilineage dysplasia ≥10% for each lineage with blast counts ≥20% without history of antecedent hematologic disease. Our analysis showed that there were no significant differences between the three AML subgroups especially when the patients were treated with hematopoietic cell transplantation (HCT).

Figure 2.

Summary/Conclusions: The long-term outcome of hypo-AML and AML-MRC were poorer than normocellular de novo AML, mainly due to older age and large proportion of adverse-risk karyotype which caused unavailable condition for HCT.

P562

INITIAL RESULTS FROM A FIRST-IN-HUMAN STUDY OF IMGN779, A CD33-TARGETING ANTIBODY-DRUG CONJUGATE (ADC) WITH NOVEL DNA ALKYLATING ACTIVITY, IN PATIENTS WITH RELAPSED OR REFRACTORY AML

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1MD Anderson Cancer Center, Houston, 2Dana-Farber Cancer Institute, Boston, 3Roswell Park Cancer Institute, Buffalo, 4University of New Mexico Cancer Center, Albuquerque, 5ImmunoGen, Inc., Waltham, 6University of Alabama at Birmingham, Birmingham, 7The Ohio State University, Columbus, 8Oregon Health and Science University, Portland, United States

Background: Acute myeloid leukemia (AML) accounts for the highest number of leukemia deaths in the United States annually. IMGN779 is an ADC that binds with high affinity and specificity to CD33, a validated therapeutic target of leukemia deaths in the United States. IMGN779 offers therapeutic potential that may overcome acquired CD33 antibody resistance.

Aims: This Phase I study is designed to establish the maximum tolerated dose (MTD) and determine the recommended phase 2 dose (RP2D) of IMGN779 when administered to patients with CD33+ AML. Evaluation of the safety, tolerability, pharmacokinetics (PK), pharmacodynamics (PD), and preliminary antitumor activity of IMGN779 are secondary objectives.

Methods: Adult patients (≥ 18 years) with relapsed or refractory CD33+ AML (defined by ≥20% of AML blasts expressing CD33 by flow cytometry) were eligible for enrollment. Informed consent was obtained from all patients. Dose escalation, which followed a standard 3+3 design, began with a starting dose of 0.02mg/kg. IMGN779 was administered intravenously once every 2 weeks on days 1 and 15 as part of a 28-day cycle. Adverse events (AEs) were evaluated using NCI-CTC v4.03.

Results: As of February 2017, a total of 17 patients (9 female, 8 male) with a median age of 62 years have received IMGN779 treatment. Five dose levels have been completed, with escalation proceeding from 0.02–0.26mg/kg. AEs were as expected for this relapsed/refractory AML population including cytopenias and constitutional symptoms. No relationship between frequency or severity...
ity of AEs and IMGN779 dose level was observed. The most common AEs were nausea (41%), febrile neutropenia (29%), and rash (29%); pneumonia, respiratory failure, and constipation were additional AEs reported in 4 or more patients (≥ 24%). The most common serious adverse events (SAEs) were grade 3 febrile neutropenia (29%) and pneumonia (24%). No dose limiting toxicities (DLTs) have been reported. Importantly, no safety signals regarding liver or hematopoietic toxicity have been observed in laboratory assessments. In general, plasma concentrations (Cmax) of IMGN779 increased relative to dose. The area under the curve (AUC) was observed in all patients through 48 hours post-infusion. A pharmacodynamics (PD) assay demonstrated consistent saturation of residual free drug. A higher general, plasma concentrations (Cmax) of IMGN779 increased relative to dose. The area under the curve (AUC) was observed in all patients through 48 hours post-infusion. A pharmacodynamics (PD) assay demonstrated consistent saturation of residual free drug.

Summary/Conclusions: This is the first clinical experience of the next generation CD33-targeting ADC, IMGN779, in AML patients. No DLTs have been noted to date. AEs were generally consistent with the underlying disease, PK and PD are favorable and dose escalation is continuing.

Aggressive Non-Hodgkin lymphoma - Relapsed/refractory

P563

COMBINATION OF TGR-1202, UBLITUXIMAB, AND BENDAMUSTINE IS SAFE AND HIGHLY ACTIVE IN PATIENTS WITH ADVANCED DLBCL AND FOLLICULAR LYMPHOMA


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Background: TGR-1202 is a next generation, once daily, PI3Kδ inhibitor, active in patients (pts) with rel/ref hematologic malignancies that has demonstrated a notably differentiated safety profile, including in long-term follow up (Burris, 2016). Ublituximab (UTX) is a novel glycoengineered mAb targeting a unique epitope on the CD20 antigen. Bendamustine (Benda) is an active chemotherapy agent in pts with lymphoma. The combination of UTX + TGR-1202 is tolerable and active in pts with rel/ref hematologic malignancies and is under Phase 3 testing for patients with CLL and Phase 2b testing for patients with DLBCL.

Aims: This Phase 1 trial evaluates the safety and efficacy of UTX + TGR-1202 + Benda in pts with advanced Diffuse Large B-cell Lymphoma (DLBCL) and Follicular Lymphoma (FL).

Methods: Eligible pts had rel/ref DLBCL or FL with an ECOG PS ≤2 w/o limit to number of prior therapies. ANC of >750 and Platelets >50,000 was permitted. Pts refractory to prior PI3Kδ, Benda, or anti-CD20 therapy were eligible. UTX was dosed on Days 1, 8, 15 of Cycle 1, Day 1 of Cycle 2-6, followed by Cycle 9 & 12. TGR-1202 was started at 800mg QD with a -1 dose reduction cohort at 600mg if not tolerated in ≥2/6 pts. Benda was dosed at 90mg/m2 on Days 1 & 2 of Cycles 1-6 only. Primary endpoints included safety and efficacy (Cheson 2007).

Results: Twenty-three pts were evaluable for safety: 15 diffuse large B-cell (DLBCL) and 8 follicular (FL). Med age 68 yo (range 31-81); 12 M/11 F; median prior treatment regimens=2 (range 1-6); 12 pts (52%) were refractory to their immediate prior treatment and to prior CD20 therapy, and 7 patients had progressed post-transplant. ECOG PS 0/1/2 (3/18/2). Initially 2/4 pts at 800mg TGR-1202 experienced AEs in Cycle 1 that led to treatment interruption (rash, neutropenia) thus the 600mg dose of TGR-1202 was explored. No additional Cycle 1 treatment delays were reported at the 600mg dose level, which was later expanded and the 800mg TGR-1202 dose is now being evaluated with stricter eligibility criteria to require an ANC of ≥1.0, and the use of growth factor support in cycle 1 is now encouraged. The most common AE’s included diarrhea (35%; G3/4 4%), decreased appetite (35%; G3/4 4%), nausea (30%; G3/4 4%), asthenia (26%; G3/4 4%) and neutropenia (22%). The only Grade 3/4 AE reported in >10% of pts was neutropenia (22%). Two pts had a TGR-1202 dose reduction. Nineteen pts (11 DLBCL/8 FL) were evaluable for efficacy: ORR amongst all pts was 79% (15/19) with 42% (8/19) achieving a complete response (CR), of which 5 were DLBCL and 3 FL. ORR in the respective groups as follows:

Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>ORR (%)</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>SD (%)</th>
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<tr>
<td>DLBCL</td>
<td>11</td>
<td>95%</td>
<td>55%</td>
<td>45%</td>
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</tr>
<tr>
<td>FL</td>
<td>8</td>
<td>88%</td>
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Median follow-up time on study is 6 mos for all pts (range 1-14+ mos).

Summary/Conclusions: The combination of UTX, TGR-1202, and bendamustine has exhibited manageable toxicity with significant activity in advanced DLBCL and FL pts including an encouraging 42% CR rate (45% in DLBCL and 38% in FL). Enrollment continues at the 800mg TGR-1202 dose level with the use of growth factor prophylaxis. Safety and efficacy data for all pts will be updated at the meeting. Based upon the early activity of the triplet, future registra-1

P564

VENETOCLAX (VEN) IN PATIENTS WITH RELAPSED/REFRACTORY NON-HODGKIN LYMPHOMA (NHL)


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Summary/Conclusions: This is the first clinical experience of the next generation CD33-targeting ADC, IMGN779, in AML patients. No DLTs have been noted to date. AEs were generally consistent with the underlying disease, PK and PD are favorable and dose escalation is continuing.

Venetoclax (VEN) is an active chemotherapy agent in pts with lymphoma. The combination of UTX + TGR-1202 is tolerable and active in pts with rel/ref hematologic malignancies and is under Phase 3 testing for patients with CLL and Phase 2b testing for patients with DLBCL.

Aims: This Phase 1 trial evaluates the safety and efficacy of UTX + TGR-1202 + Benda in pts with advanced Diffuse Large B-cell Lymphoma (DLBCL) and Follicular Lymphoma (FL).

Methods: Eligible pts had rel/ref DLBCL or FL with an ECOG PS ≤2 w/o limit to number of prior therapies. ANC of >750 and Platelets >50,000 was permitted. Pts refractory to prior PI3Kδ, Benda, or anti-CD20 therapy were eligible. UTX was dosed on Days 1, 8, 15 of Cycle 1, Day 1 of Cycle 2-6, followed by Cycle 9 & 12. TGR-1202 was started at 800mg QD with a -1 dose reduction cohort at 600mg if not tolerated in ≥2/6 pts. Benda was dosed at 90mg/m2 on Days 1 & 2 of Cycles 1-6 only. Primary endpoints included safety and efficacy (Cheson 2007).

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Median follow-up time on study is 6 mos for all pts (range 1-14+ mos).

Summary/Conclusions: The combination of UTX, TGR-1202, and bendamustine has exhibited manageable toxicity with significant activity in advanced DLBCL and FL pts including an encouraging 42% CR rate (45% in DLBCL and 38% in FL). Enrollment continues at the 800mg TGR-1202 dose level with the use of growth factor prophylaxis. Safety and efficacy data for all pts will be updated at the meeting. Based upon the early activity of the triplet, future registration directed studies are being planned.
**Background:** VEN is a selective orally bioavailable BCL-2 inhibitor. The dose-escalation Phase 1 study of VEN in 106 patients (pts) with relapsed/refractory NHL reported an ORR of 44%. Most pts had diffuse large B-cell/follicular lymphoma.

**Aims:** We report on updated results in pts with less common NHL subtypes.

**Methods:** VEN was administered and continued until progressive disease (PD) or unacceptable toxicity, in dose cohorts ranging from 300-1200mg. Adverse events (AEs) were assessed by NCI-CTCAE v4.0 and response by 2007 Cheon ISG response criteria, utilizing CT scans beginning at wk 6.

**Results:** 35 of 106 pts had mantle cell lymphoma (MCL, n=28), marginal zone lymphoma (MZL, n=5) or Waldenström macroglobulinemia (WM, n=4). Most common unacceptable adverse events (AE) were nausea (51%), diarrhea (49%) and fatigue (34%); grade 3/4 AEs in >10% of pts were neutropenia and anemia (17% each). Laboratory TLS was reported in a single pt (bulky MCL). MCL pts (median age: 72 years) had received a median of 3 (1-7) prior treatments (tx). Median time from start of prior tx to start of VEN was 13 mo (2-148) and time on VEN was 42, 17, 54, 20 mo. All pts achieved PR (at wks 6 [n=2], 16 and 36), with DORs of 11, 12, 38 and 50+ mo (latter is ongoing and remains on study).

**Summary/Conclusions:** At 0.2-42. In DLBCL, cell of origin (IHC) did not influence continuation were progression (35%), toxicity (20%), and bridge to CAR-T (10%). Median number of prior therapies was 3 (range 0-11). All RT pts were not treated with IBR previously for CLL. The three most common reasons for IBR discontinuation were progression (35%), toxicity (20%), and bridge to CAR-T (10%). PFS and OS data are shown in Table 1. In DLBCL, cell of origin (IHC) did not impact outcomes (p=0.97, LR test). Patients with RT had better PFS as compared to de novo DLBCL (p=0.03, LR test).

**Table 1.**

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<td><strong>Summary/Conclusions:</strong></td>
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<td><strong>WHOLE BODY DIFFUSION-WIGHTED MAGNETIC RESONANCE IMAGING IS A GOOD PROGNOSTIC TOOL FOR TREATMENT OUTCOME AFTER ONE CYCLE OF IMMUNOCHEMOTHERAPY IN AGGRESSIVE LYMPHOMA</strong></td>
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<td>1Radiology, 2Nuclear Medicine, 3Medical Oncology, 4Hematology, University Hospitals Leuven, Leuven, Belgium</td>
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<td><strong>Background:</strong> Early identification of non-Hodgkin lymphoma patients not responding to therapy may enable treatment adaptation which might impact on outcome. The use of diffusion-weighted imaging (DWI) and ADC maps to assess for treatment response has been reported, but a large-scale study analyzing homogenous patient population is lacking. Here we report on the first large scale study of 50 pts with aggressive NHL, treated with VEN as monotherapy in the RR setting.</td>
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<td><strong>Aims:</strong> To further characterize the efficacy of IBR in patients with RR DLBCL, Richter’s transformation (RT) or FL. <strong>Methods:</strong> We conducted a retrospective cohort study of DLBCL, RT and FL patients consecutively treated with IBR. Data collected included patient demographics, stage, IPI, genetic characteristics, prior treatments, IBD dose and discontinuation reasons, and response. PFS and OS were estimated using the Kaplan-Meier method and survival analysis by the log rank (LR) test.</td>
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<td><strong>Results:</strong> 44 patients were identified (DLBCL: n=24, 54.5%; FL: n=12, 27%; RT: n=18, 18%) who received IBR monotherapy in the RR setting. Baseline characteristics included age (range 19–80), 61% male, 95% ECOG 0 - 1, 71% stage IV, 62% elevated LDH, and 48% R-IPI ≥ 4. DLBCL sub-types (Hans criteria) were 25% non-GC (n=11), 16% GC (n=7), and 14% unclassifiable (n=6). In the FL subgroup, 8% were grade 1, 58% were grade 2, 33% were grade 3a. Median number of prior therapies was 5 (range 1-11). All RT patients were not treated with IBR previously for CLL. The three most common reasons for IBR discontinuation were progression (35%), toxicity (20%), and bridge to CAR/T (10%). PFS and OS data are shown in Table 1. In DLBCL, cell of origin (IHC) did not impact outcomes (p=0.97, LR test). Patients with RT had better PFS as compared to de novo DLBCL (p=0.03, LR test). <strong>Summary/Conclusions:</strong> In the largest single-center, real-world experience of IBR use in DLBCL, RT and FL, we validate findings reported in clinical trials. In FL, responses appear to be durable (median PFS of &gt;10 months). Outcomes are extremely poor in DLBCL and use of IBR as monotherapy is not recommended. Perhaps IBR is best used as a short-term bridge to more definitive therapies. Cell of origin by immunohistochemistry does not predict PFS and should not be used to preferentially select non-GC DLBCL patients for IBR. Patients with RT appear to have more durable responses (vs DLBCL) suggesting differing dependence on BTK signaling for tumor survival.</td>
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<td><strong>PREVALENCE AND PROGNOSTIC VALUE OF MYD88 AND CD79B MUTATIONS IN IMMUNE-PRIVILEGED SITE AND (EXTRA)NODAL DLBCLS</strong></td>
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<td><strong>Background:</strong> From a biological point of view, immune-privileged sites (IPS) [1] are defined as sites where immune mechanisms are compromised and provide a protective effect for tumor cells. This effect is mediated by the presence of bone marrow stromal cells and B cells. As a result of impaired immune surveillance, extranodal disease is found in up to 20% of cases. The mutational status of MYD88 and CD79B genes is of great interest, as both genes are known to play a role in the regulation of the immune response.</td>
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| **Aims:** To assess the prevalence and prognostic value of MYD88 and CD79B mutations in IPS and extranodal DLBCL. **Methods:** We performed DNA sequencing for MYD88 and CD79B mutations in bone marrow, peripheral blood, and lymph node samples collected from 35 patients with IPS or extranodal disease at our institute. Mutational analysis was performed using Sanger sequencing and direct PCR. **Results:** The prevalence of MYD88 and CD79B mutations was highest in IPS (24%) and extranodal disease (20%). The most common mutation was the MYD88 V600E mutation, which was found in 16% of IPS samples and 12% of extranodal samples. The CD79B mutations were found in 8% of IPS samples and 12% of extranodal samples. **Summary/Conclusions:** The prevalence of MYD88 and CD79B mutations in IPS and extranodal DLBCL is high, with the MYD88 V600E mutation being the most common. These mutations are potential therapeutic targets and may be used as prognostic biomarkers.**
Background: Activating mutations in CD79B and MYD88 are important molecular drivers of a subset of diffuse large B-cell lymphomas (DLBCLs), activating the B-cell receptor and toll-like receptor pathways, respectively. Interestingly, the frequency of these mutations differs greatly among DLBCLs at different anatomical sites, with a remarkably high prevalence at immune-privileged (IP) sites (central nervous system and testis). Recent studies suggest that these mutations are associated with an unfavorable prognosis. However, the prognostic value in relation to the site of presentation has not yet been explored.

Aims: To investigate if mutations in MYD88 and CD79B are independent prognosticators for overall survival (OS) in DLBCL, particularly in patients with lymphomas at IP sites, for which a high prevalence of these mutations was reported.

Methods: In this retrospective study, we investigated a large clinically annotated cohort of 189 consecutive primary DLBCLs, including primarily nodal (N=64), primarily extranodal (N=74) and IP localizations (N=51). Patients were diagnosed between 1990-2015 at the Academic Medical Center, (University of Amsterdam) or other Dutch hospitals. The vast majority was treated with (R-)CHOP (N=143) or other immune-chemotherapies (N=16). Detailed clinical characteristics of all patients were collected. For all patients BCL2, BCL6, and MYC translocations, Epstein Bar Virus (EBV) status and the mutational status of MYD88 and CD79 were assessed, employing methods described previously (Kraan et al., BCJ 2013).

Results: Translocations in BCL2, BCL6 and MYC were identified in 14, 32 and 13 patients, respectively and 23 EBV-positive cases were found. MYD88 and CD79 were assessed, employing methods described previously.

Background: Activating mutations in CD79B and MYD88 are important molecular drivers of a subset of diffuse large B-cell lymphomas (DLBCLs), activating the B-cell receptor and toll-like receptor pathways, respectively. Interestingly, the frequency of these mutations differs greatly among DLBCLs at different anatomical sites, with a remarkably high prevalence at immune-privileged (IP) sites (central nervous system and testis). Recent studies suggest that these mutations are associated with an unfavorable prognosis. However, the prognostic value in relation to the site of presentation has not yet been explored.

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Background: Nodal pcral T-cell lymphomas (PTCLs) are a heterogeneous group of neoplasms, which include pcrAL not otherwise specified (pctL-NOS), anciogloinflammatory T-cell lymphoma (AITL), anciogpaic large-cell lymphoma, analopic lymphoma kinase positive (ALK-ALK+), and ALCL-ALK-. Clinical assessments before and after treatment are essential to predict survival in pctL-NOS. However, limited data is available regarding the prognostic relevance of National Comprehensive Cancer Network International Prognostic Index (NCCN-IPI) and post-treatment PET-CT scan.

Methods: In this retrospective cohort study, patients with newly diagnosed pctL-NOS were consecutively enrolled from 11 hospitals in South Korea. Patients were eligible if they were histologically diagnosed with nodal pctL-tcl from Jan 2005 to June 2016, received systemic chemotherapy, and had the results of PET-CT scan at the time of diagnosis and at the end of treatment. Post-treatment PET-CT was assessed using 5-point Deauville score. The study excluded ALCL-ALK+ due to well-known better survival.

Results: A total of 396 patients were screened for eligibility. Seventy patients were excluded from the analysis due to following reasons: unavailable pre- or post-treatment PET scans, no systemic treatment, uncertain histology, and post-treatment PET-CT scan indicating tumor viability.

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Results: A total of 396 patients were screened for eligibility. Seventy patients were excluded from the analysis due to following reasons: unavailable pre- or post-treatment PET scans, no systemic treatment, uncertain histology, and post-treatment PET-CT scan indicating tumor viability.
Summary/Conclusions: Crizotinib confirmed to be an effective and safe therapy for advanced relapsed ALK+ALCL with durable responses up to 6 years after treatment initiation and no relapse later than 4 months. These results represent the longest available safety record for crizotinib. ALK point mutations can develop and 2nd/3rd generation inhibitors may be a therapeutic opportunity for patients who develop resistance to crizotinib.

P571
PRELIMINARY RESULTS FROM AN OPEN-LABEL, PHASE II STUDY OF TIPIFARNIB IN RELAPSED OR REFRACTORY PERIPHERAL T-CELL LYMPHOMA
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1Mayo Clinic, Rochester, 2H. Lee Moffitt Cancer Center & Research Institute, Tampa, 3Dana Farber Cancer Institute, Boston, 4Stanford University Medical Center, Stanford, United States, 5DIVAL Instituto de Investigación Marqués de Valdecilla, Santander, 6Fundación Jiménez Díaz, Madrid, Spain, 7Kura Oncology, La Jolla, United States

Background: Tipifarnib is a potent and selective inhibitor of farnesyltransferase (FT). FT catalyzes post-translational attachment of farnesyl groups required for localization of signaling molecules to the inner cell membrane. CXCL12 is a chemokine that is essential for hematopoietic stem cell (HSC) homing to the bone marrow and lymphoid organs and for maintenance of HSCs and immune cell progenitors. CXCL12 is known to signal in part through HRAS, a signaling protein that is uniquely farnesylated. Tipifarnib has previously been shown to be well tolerated and to have a 41% response rate (7 responses out of 17 patients) in patients (pts) with T-cell Non-Hodgkin Lymphoma, including 4 objective responses in 8 pts with peripheral T-cell lymphoma (PTCL) (Witzig et al, 2011). Building on this prior experience, we report herein the preliminary efficacy, safety and biomarker data from our ongoing Phase 2 study in PTCL.

Aims: This Phase 2 study is a multi-institutional, single-arm, open-label, two-stage (11+7) study designed to determine the efficacy and safety of tipifarnib in pts with relapsed/refractory (R/R) PTCL.

Methods: Pts with R/R PTCL after prior cytotoxic systemic therapy, aged ≥ 18 years old, and with a performance status of 0-2 were eligible. Informed consent was obtained. The following subtypes of PTCL were eligible for enrollment: PTCL not otherwise specified (PTCL-NOS), angioimmunoblastic T-cell lymphoma (AITL), ALK-positive and -negative anaplastic large cell lymphoma (ALCL), hepatosplenic T-cell lymphoma, enteropathy-associated T-cell lymphoma (EATL), extranodal natural killer (NK)-T cell lymphoma, nasal type and subcutaneous panniculitis-like T-cell lymphoma. The primary endpoint of the study is overall response rate. Secondary endpoints include safety and tolerability, duration of response (DOR) and progression free survival (PFS). Based on activity observed in the first 18 pts in the study, the protocol has been amended and enrollment is ongoing to an expansion cohort in AITL (N=12). Enrolled pts are treated with tipifarnib 600mg administrated orally twice daily on days 1-7 and 15-21 of 28-day treatment cycles until progression of disease or unacceptable toxicity. Biomarker studies included gene expression profiling of pre-treatment tumor biopsies by RNASeq and DNA next-generation sequencing (NGS). Clinical trial information: NCT02464228.

Results: At data cut-off (2/15/2017), 18 pts (2 ATL, 1 ALK-ALCL, 15 PTCL-NOS) were treated with tipifarnib. Most common treatment-related AEs (grade ≥ 3) were myelosuppression, including neutropenia (61%), anemia (39%) and thrombocytopenia (39%). 3 pts achieved a partial response (2 ATL; 1 PTCL-NOS) and 3 additional pts experienced stable disease >6 months. Tumor DNA from 18 pts was sequenced using NGS, 8 pts of 18 were sequenced using CXLCL12 3'UTR single nucleotide variation (SNV) was observed. 7 of 16 pts carried the rs2839695 variant while an additional patient carried a novel variant. The presence of 3'UTR SNVs was associated with low levels of CXLCL12 gene expression and disease progression. Figure 1 shows all pts deriving clinical benefit from tipifarnib in the designated reference (wild-type) 3'UTR CXLCL12 and had tumors that expressed high levels of mRNA for this chemokine. Testing of circulating CXLCL12 levels is ongoing.

Summary/Conclusions: Although this study is ongoing, these preliminary data indicate that tipifarnib is generally well-tolerated and has antitumor activity, particularly in pts with AITL histology, absence of 3'UTR CXCL12 SNV and high levels of CXCL12 gene expression.

Figure 1.

P572
BAM CONDITIONING BEFORE AUTOLOGOUS STEM CELL TRANSPLANTATION FOR LYMPHOMA: A RETROSPECTIVE STUDY ON BEHALF OF THE FRANCOPHONE SOCIETY OF BONE MARROW TRANSPLANTATION AND CELLULAR THERAPY (SFGM-TC)
1Service d’hématologie, 2Département d’hématologie, Institut de Cancérologie de la Loire, Saint-Etienne, 3Service d’hématologie, CHU de Clermont-Ferrand, Clermont-Ferrand, 4Service d’hématologie, Centre Hospitalier Universitaire, Besançon, 5Service d’hématologie, Centre Hospitalier Universitaire, Lyon, 6Service d’hématologie, Centre Henri Becquerel, Rouen, 7Service d’hématologie, Centre Léon Bérard, Lyon, 8Service d’hématologie, CHU d’Angers, Angers, 9Service d’hématologie, CHU de Tours, TOURS, 10Service d’hématologie, Centre Léon Bérard, Lyon, 11Service d’hématologie, CHU de Dijon, Dijon, 12Service d’hématologie, Centre hospitalier universitaire, Nice, 13Service d’hématologie, CHU de Toulouse, Toulouse, 14Service d’hématologie, CHU de Reims, Reims, 15Service d’hématologie, CHU de Metz, Metz, 16Service d’hématologie, CHU de Dijon, 17Service d’hématologie greffe, Hôpital Saint-Louis, APHP, PARIS, 18CHU de Tours, Centre Hospitalier Universitaire de Tours, Tours, France

Background: High-dose chemotherapy before autologous stem cell transplantation (ASCT) is a therapeutic option as a consolidation in primary or relapsed lymphoma. BEAM conditioning is generally used. Alternative conditioning regimens have been published but few data are available.

Aims: To evaluate tolerance and efficacy of the BAM (Busulfan, AraCytin and Melphalan) conditioning before ASCT.

Methods: We conducted a retrospective study in 188 French patients treated between 2000 and 2015. Data were retrospectively collected from the Promesse database, an informed consent was obtained from all patients.

Results: Indications for ASCT were diffuse large B-cell lymphoma (n=54, 29%), mantle-cell lymphoma (n=42, 22%), Hodgkin’s disease (n=33, 18%), low-grade non-hodgkin lymphoma (n=26, 14%), T-cell lymphoma (n=17, 9%), Burkitt’s lymphoma (n=8, 4%) and B-cell lymphoma (n=8, 4%). Median age at diagnosis was 50.9 years (35-59). Time between diagnosis and ASCT was 295 days (176-777). Patients received 1 (n=82, 44%), 2 (n=83, 44%), 3 or more (n=18, 10%), unknown (ND) (n=5, 2%) treatment lines before ASCT. Among the 138 B-cell lymphoma patients, 132 received rituximab before ASCT. Only 20 patients received prior radiation therapy. In all patients, ASCT was the first transplantation. In 11 patients, ASCT was planned as part of a multiple graft protocol. At the time of transplantation, 116 (62%) patients were in complete remission, 54 (29%) in partial remission, 13 (7%) in relapse or progression, and 5 (2%) ND. ASCT was documented in 186 (99%) patients. Median time to neutrophil engraftment was 11 days [10-12] and 19 days [14-32]. Infectious complications were found in 153 patients. One hundred (53%) patients had undocumented fever, 19 (10%) had sepsis, 150 (80%) had grade 1-4 mucositis during neutropenia with a WHO toxicity grading of 2 (42%), 3 (39%) and 4 (19%). Colitis with a median duration of 7 days [5-10], was reported in 73 patients, with a maximum toxicity grading of 1-2 (n=43, 59%), 3 (n=21, 29%) or 4 (n=4, 6%) and ND in 5 patients. Only 2 (1%) patients had non-fatal hepatic sinusoidal obstruction syndrome. Pulmonary toxicity was reported in 33 (17.6%) patients with 8 cases of respiratory distress syndrome. Respiratory distress was fatal in one patient but occurred more than 6 months after ASCT and salvage treatment. Seven (3.7%) patients patients reported secondary cancers (all were solid tumors except one acute leukemia). Median follow-up was 17.1 months [11.3-29.5]. At the time of the study, 47 (25%) patients had relapsed. Cumulative incidence of relapse was 6.24% at 3 months and 17.31% at 12 months. At the end of the follow-up, 149 (79%) patients were alive. The main causes of death were relapse (n=15, 41%) and toxicity (n=16, 43%). Median overall survival (OS) was not reached and progression-free survival was 71.5 months [47-71]. Relapse-free mortality was 1.66% at 3 months and 4% at 12 months. In the univariate analysis, the number of treatment lines (1 or 2) before ASCT and previous use of monoclonal antibodies positively impacted the OS. Conversely, the multiple graft protocol had an unfavourable impact on OS.

Summary/Conclusions: BAM conditioning before ASCT for lymphoma helps to control disease activity without excessive toxicity. It may be a suitable alternative to BEAM in case of drug shortage. However, comparative studies are needed to confirm these findings.
Bone marrow failure syndromes incl. PNH - Clinical

P573
ANALYSIS OF MICRORNAOME, PROTEOME AND METABOLOME OF EXOSOMES FROM PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a clonal disease caused by the lack of glycosyl asialo phosphoryl proteins at the cell membrane that leads to intravascular hemolysis upon complement activation. Patients have intravascular haemolysis with high risk of thrombosis, and a variable degree of bone marrow failure. Treatment with Eculizumab reduces intravascular hemolysis and also the thrombotic risk. The mechanism of thrombosis in PNH is still unknown. Exosomes are produced by many cells and whose secretion is closely related with the inflammatory status. Exosomes participate in cell communication by activating signaling pathways and transferring genetic material, i.e. miRNA, and proteins to host cells.

Aims: To describe the microRNAome, proteome and metabolome of exosomes from PNH patients to identify potential biomarkers of the disease and to investigate its relationship with the mechanism of thrombosis in these patients.

Methods: Plasma exosomes were isolated from 5 healthy controls and from 9 PNH patients(6 with Eculizumab, 3 with thrombosis –ET- and 3 without thrombosis –ENT- and 3 without Eculizumab) using Total Exosomesolation kit (ThermoFisher). miRNAs from exosomes were purified using Nucleo Spin miRNA Plasma Kit (Macherey-Nagel). miRNA expression was evaluated by plasma/serum focus miRNAs PCR panel V (Exiqon). Proteomic analysis of exosomes was performed at the OMIcs core facilities. Untargeted metabolomic analysis was performed by using combination of gas chromatography and liquid chromatography (LC) with mass spectrometry (MS). Additionally, latest advances were used combining LC-MS-solid phase extraction-nuclear magnetic resonance (UPLC-QTOF_SPE_NMR) ‘on line’ for unequivocal structural elucidation of unknown metabolites.

Results: Mir-16-5p and miR-451a had lower levels in patients vs controls. Eculizumab treatment increased their expression, particularly in the group with thrombosis. Eculizumab also decreased miR-223-3p (the most abundant miR and the most differentially expressed between patients and controls) and with that has been associated with its activity)and increased miR-15a-5p levels (0.50- and 3.12-fold respectively). Those proteins differentially expressed in patients and controls were related with the complement system and the immune response. We identified an increase in the plasma hemoglobin levels in patients vs controls (4.9-fold), which is related with platelet activation. It is also noteworthy the decrease (1.5-fold) of the anticoagulant Protein S in patients vs controls. When the analysis was performed among the 3 groups of patients, only Ig heavy chain V4-1 region HG3 increased in 3.9-fold in the Eculizumab group vs without Eculizumab group, which could be related with the treatment. We identified quite few metabolites inside the exosomes, all of them associated with cell toxicity or immune response. The levels of Cholesterol, HydroxyTerbinafine-glucuronide and Diacyl-glycerol decreased in 17.3, 17.6 and 19.4-fold, respectively in patients treated with Eculizumab. Interestingly, the Aminoethylphosphonicacid, Cholesterol and PGF2 increased 16.7-, 21- and 19.4-fold in patients with thrombosis.

Summary/Conclusions: Our study supports that exosomes contain material that may influence the pathological status of the PNH patients. In concordance, most of the proteins, miRNAs and metabolites are related with the complement system or the inflammatory response. In future experiments, some of the proteins, miRNAs and metabolites should be validated to define whether they could be considered biomarkers.

P574
Abstract withdrawn.

P575
SEVERE CHRONIC NEUTROPENIA: THE ROLE OF PRIMARY IMMUNODEFICIENCY AS CAUSATIVE AGENTS. A SINGLE CENTER DATA

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Background: Severe Chronic Neutropenia may be a primary disease, usually defined as congenital (CN), or a condition mainly secondary to autoimmune disturbances (SN) (1,2). CN rises in early infancy, has a narrow block at pro/myelocyte, classically carries genes ELANE/HAX1 mutations in 70% of cases and is G-CSF dependent. SN is accompanied by extrahaematological signs and/or positivity of autoimmune markers; bone marrow has a normal morphology or is “left shifted”. In spite of these categorization many cases do not fit one group and share features of both of them. These “ovelap Neutropenia” (ON) patients are a diagnostic and management challenge.

Aims: Investigate the genetic background of this ON from a cohort of chronic neutropenia subjects screened at Hematology Unit of Gaslini Hospital and characterize their clinical phenotype.

Methods: Patients with severe chronic neutropenia were seen prospectively in our center and diagnosed/followed-up according to published guidelines(3,4).

Genetic diagnosis includes classical Sanger technique foe commonest severe chronic neutropenia genes and an enlarged NGS panel including also those gene responsible for PID.

Results: From 2008 to 2016, 24 patients (13 males) with median age at last follow of 18yrs (range 28 mo-51y) had a complete work up for severe chronic neutropenia (Table 1). Ten/24 subjects (43%) were diagnosed as classical CN with ELANE mutation found in the majority (80%) of cases. Seven/24 (29%) were diagnosed as SN and the remaining 7/24 (29%) as a PID. Eculizumab mutation was found in a total of 8/24 patients (30%) with 7 SN subjects (71%) and 3 to the 7 ON subjects (42%).Table 1 shows clinical hematological characteristic of the 3 categories of patients.

Summary/Conclusions: A considerable portion (30%) of subjects affected with severe chronic neutropenia have been identified as PID. In the group of ON subjects a mutated PID gene was found in 3/7 patients and mutations of ELANE in 2/7 patients. No mutation was found in the remaining 2. The phenotype of ON subjects is characterized by extra-hematological autoimmune symptoms, by maturation block and by the frequent involvement of more than one hematopoietic lineage. This phenomenon may suggest to access to an enlarged genetic panel including PID genes for genetic diagnosis. An accurate immunological and genetic work may support diagnosis and management of these difficult patients.

Table 1.

References

P576
TREATMENT WITH HORSE-DERIVED ANTI-ThYMOCYTE GLOBULIN IS NOT ENOUGH TO ENDURING HEMATOLOGICAL RESPONSES AND A 1.5 YEAR SURVIVAL PROBABILITY OF 87% IN ADULT ACQUIRED APLASTIC ANEMIA PATIENTS IN THE NETHERLANDS

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IMMUNE RECONSTITUTION IN PATIENTS WITH ACQUIRED SEVERE APLASTIC ANEMIA AFTER HAPLOIDENTICAL STEM CELL TRANSPLANTATION

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Background: Acquired severe aplastic anemia (SAA) is a rare disease that is characterized by bone marrow failure that results in pancytopenia and hypocellularity of the bone marrow. Hematopoietic stem cell transplantation (SCT) is one of the main treatment strategies for SAA. In recent years, for patients who require transplantation, but have no human leukocyte antigen (HLA) matched donors, haploidentical SCT (haplo-SCT) is an important alternative option. Delayed immune reconstitution (IR) after haplo-SCT played a crucial role in infection and other transplant-related diseases (TRD), and was considered as a barrier to the wider application of haplo-SCT in SAA. The assessment of immune reconstitution may provide tools to better predict and modulate adverse outcomes and consequently improve survival after transplantation. However, the kinetics of immune recovery in patients with SAA after haplo-SCT have not been studied systematically.

Aims: We aim to provide the kinetics for immune reconstitution in SAA patients who receive haplo-SCT, investigate the factors that may affect immune recovery and assess the impact of immune cell subset recovery on transplant outcomes.

Methods: In this study, we examined immune cell subset counts and immunoglobulins in 81 SAA patients from day 30 to day 365 after haplo-SCT. The immune cells analyzed in this study including lymphocyte, monocyte, CD3+ T cell, CD8+ T cell, CD4+ CD8- T cell, CD4+CD8+ T cell, CD4+ CD28+ T cell, CD4+ memory T cell and CD4+ naïve T cells. Simultaneously, we determined which factors influence immune reconstitution and analyzed the impact of immune cell subsets on transplant outcomes.

Results: (i) The reconstitution of different immune cell subsets occurred at different rates after haplo-SCT. Monocytes were the first to recover, followed by CD8+ T and CD19+ B cells, and finally CD4+ T cells. Early CD4+ T cell recovery occurred at the expense of memory cells, whereas naive CD4+ T cells rose only 9 months after SCT. (ii) In the multivariate analysis, lower recipient age, female gender, high mononuclear cell counts and CD4+ T cell counts in the graft were associated with improved immune recovery after transplant. (iii) A CD4/CD8 ratio less than 0.567 on day 30 post-transplantation was associated with lower treatment related mortality and higher overall survival after haplo-SCT in SAA patients.

Summary/Conclusions: We provided the kinetics for immune recovery in SAA patients who received haplo-SCT. In general, our study demonstrated that the recovery of monocyte and CD8+ T cells was fast in SAA patients, whereas the recovery of the CD4+ T cell subset was delayed. In addition, our data suggested that the CD4/CD8 ratio may be useful for predicting transplant outcomes in SAA patients after they complete haplo-SCT. Our results may be useful for making better predictions and modulating the IR of SAA patients, which would subsequently improve the outcomes after transplantation.
Dutch pediatric DBA population, limitations of our study include a relatively small number of patients, and the lack of complete genetic analysis (for all DBA candidate genes) in a relevant number of patients. Overall, to increase our understanding of genotype-phenotype correlation in DBA, and underlying pathophysiological mechanisms more generally, it crucial to further extend our genetic, and functional analysis of DBA-candidate genes, as well as compare, and share data from international registries.

**P579**

**DIAMOND-BLACKFAN ANEMIA IN THE NETHERLANDS: AN OVERVIEW OF CLINICAL CHARACTERISTICS AND UNDERLYING MOLECULAR DEFECTS**

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1Pediatric Haematology, clinical research, 2Pediatric Haematology and Stem Cell Transplantation, Wilhelmina Children’s Hospital, Utrecht, 3Euro DBA, genetics, and functional analysis of DBA-candidate genes, as well as compare, and share data from international registries. Overall, to increase our understanding of genotype-phenotype correlation in DBA, and underlying pathophysiological mechanisms more generally, it crucial to further extend our genetic, and functional analysis of DBA-candidate genes, as well as compare, and share data from international registries.

**Methods:** Twelve Delphi panelists from 6 countries, all of whom were clinicians with expertise in PNH, were recruited. Consensus was reached on 22 of 23 PNH screening and diagnostic decision points identified by the Delphi panelists. Specifically, consensus was gained on the core symptoms and signs of PNH at presentation, including hemolysis, bone marrow dysfunction, and thrombosis. Consensus was not reached for 36 of 38 key screening and diagnostic tests required at each decision point to narrow the differential diagnosis and to confirm the diagnosis of PNH. The level of agreement on screening and diagnostic decision points and tests was sufficient to enable the development of a screening and diagnostic algorithm (Figure) that is consistent with the published literature and with the real-world experience of the international expert advisory committee.

**Summary/Conclusions:** The modified Delphi methodology facilitated development of a consensus-based, clinically relevant PNH screening and diagnostic algorithm. This algorithm provides clinicians with varying levels of expertise detailed guidance on how to screen for and diagnose PNH.

**P580**

**NEXT GENERATION SEQUENCING IN BONE MARROW FAILURE SYNDROMES**

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**Background:** Inherited bone marrow failure syndromes (IBMFSs) are a heterogeneous group of genetic disorders, with similar clinical presentations, resulting in complex diagnosis. Molecular characterization is essential in order to establish diagnosis, treatment and prognosis. Next-generation sequencing (NGS) techniques seem to be a useful platform for genetically defining different IBMFSs.

**Aims:** To design a NGS panel with the objective of making a specific, fast and cost-effective diagnosis for these pathologies.

**Methods:** We developed a NGS panel of 164 genes involved in different IBMFSs. A total of 120 samples have been processed. Patients were classified into two groups based on the NGS results: classified IBMFSs (CBMFS) for those with a clinical picture typical of some of these disorders, and unclassified IBMFSs (UBMFS) for the others. For the NGS study the NextSeq platform of Illumina (Roche) has been used. Bioinformatic analysis has been oriented to the identification of point polymorphisms (SNPs) and insertions/deletions of small DNA fragments.

**Results:** Of the 120 samples processed, 10% (12/120) was not suitable for analysis. A total of 108 patients were studied. In 59.3% (64/108) causal mutations were detected. From the total samples analyzed (108), 75% (81/108) were included in the CBMFS patient group, obtaining a diagnostic yield of 64.2% (52/81). The remaining 27 patients (25%) were included in the UBMFS group and we found causal mutation in 37% (10/27). Therefore, it remains a percentage of patients without a genetic diagnosis, which seems more evident in the UBMFS group. This could be explained by the fact that the causal gene has not been described or due to the limitations of the technique.

**Summary/Conclusions:** NGS techniques are a fast and cost-effective option for the diagnosis of IBMFSs patients. In our series, we have reached a diagnosis rate of 93.3%, coinciding with that described in the literature. Undiagnosed patients should be included in new research projects.

**P581**

**APLASTIC ANEMIA PATIENTS WITH MONOCYTE-DOMINANT PNH CLONES HAVE A UNIQUE PRESENTATION AND ARE LESS RESPONSIVE TO IMMUNOSUPPRESSIVE THERAPY**

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**Background:** Aplastic anemia (AA) is a bone marrow failure syndrome that can be successfully treated with either immunosuppressive therapy (IST) or allogeneic bone marrow transplantation (BMT). In ~50% of patients (pts) with AA, a clone deficient in glycosylphosphatidyl inositol (GPI)-linked antigens—a paroxysmal nocturnal hemoglobinuria (PNH) clone—can be detected (Young, Blood, 2006). In recent years, highly sensitive techniques have been developed to test for PNH clones that have primarily focused on evaluating peripheral blood white cells. Neutrophils are routinely tested for expression of GPI with fluorescent aerosol (FLAE); monocytes may also be analyzed but are not always examined in PNH testing. Our centre has previously reported that 60% of PNH-positive tests show a higher monocyte clone than granulocyte clone and that there was >10% difference in 20% of these discrepant results (Razavi, ISHL Proceedings, 2015). Whether pts with discordant monocyte and granulocyte PNH clones have different clinical characteristics and/or response to IST has not been reported to date.

**Aims:** To compare the granulocyte and monocyte PNH clones in pts with AA to determine whether there are differences in clinical presentation and/or response to IST for pts with discordant clone sizes.

**Methods:** A retrospective review was performed on all patients >16 treated with IST at VGH, the tertiary referral centre for the Province of BC, between 11/09 and 10/15. All patients had central pathology review and metaplastic cytogenetic analysis that confirmed a diagnosis of AA. High-sensitivity flow cytometry testing with a sensitivity of 0.1% was done on all patients

haematologica | 2017; 102(s2) | 225

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to detect the presence of a PNH clone. Granulocytes, monocytes and erythrocyes were integrated with multi-colour flow panels including CD64 and FLAER. The criteria for determining discordant granulocyte and monocyte clone sizes was dependant upon the absolute size of the smaller clone. For clones 0.1-10%, discordance was defined as when the larger clone either ≥2 x the smaller clone or at least 1% (absolute value) greater. For smaller clones >10%, the larger clone had to be ≥110% its size. IST was uniform - Cyclosporine (CSA, 2.5mg/kg p.o. b.i.d.), anti-thymocyte globulin (ATG; ATGAM® 40mg/kg IV daily x 4 days) and (Methylprednisolone 1mg/kg/day x 10 days). CSA doses were adjusted to maintain whole blood trough CSA level of 200-300 μg/L for 12-months followed by slow taper based upon hematologic response. Non-responders at 6 months were eligible to proceed to either a second cycle of ATG or BMT, if a suitable donor was available. Severity of AA [very severe (VSAA), severe (SA) or non-severe (NSA)] and response to IST [(none, partial (PR) or complete (CR)] were determined according to published criteria (Marsh, Br J Haematol, 2009). Statistical comparisons were done using a standard Chi square analysis.

Results: 30 pts with AA and a PNH clone were identified, 18 females and 12 males with median age of 50.5 years (range 17-71). There were 14 pts with NSA, 13 with SAA and 3 with VSAA. Responses were seen in 20/30 pts (66.7%) including 13 PR and 7 CR. Six pts relapsed with CSA tapering and 5 responded to intensified IST. 2 pts required Eculizumab after evolving to a classic PNH phenotype. Six pts underwent BMT for primary non-response and 4 pts have died (2 post-BMT, 1 from complications of AA and 1 from breast Ca); 26 pts remain alive and well with a median follow-up of 48 mos (15-86). There were 17 pts (56%) with concordant granulocyte and monocyte clone sizes (Group 1), 4 pts (13%) had granulocyte-dominant disease (Group 2) and 9 pts (30%) had monocyte-dominant disease (Group 3). Group 3 pts were significantly more likely to have NSA and showed a trend toward an inferior response rate to IST (Table 1).

Summary/Conclusions: Our data are able to independently confirm the findings of previous studies concerning hematologic recovery rates in pts with primary AA following IST with ATG by providing further evidence that rATG plus CsA is inferior to hATG plus CsA when administered as a first-line treatment. In addition, we were able to observe in pts ≤50 yrs, irrespective gender, an overall higher hematologic recovery. For this reason, it remains unclear why ATGAM® is still not approved in Germany as first-line therapy in pts with AA, as the only hATG product registered in Europe (Lymphoglobulin®) was withdrawn from the market in 2007.

Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>p-value</th>
<th>Response rate (%)</th>
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<td>Group 1</td>
<td>10/17</td>
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<td>Group 2</td>
<td>4/4</td>
<td>6/17 (35%)</td>
<td></td>
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<tr>
<td>Group 3</td>
<td>2/9</td>
<td>0/7 (0%)</td>
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**P582 RESPONSE TO ANTI-THYMOCYTE GLOBULIN (ATG) IN PATIENTS WITH APLASTIC ANEMIA (AA): A SINGLE-CENTRE EXPERIENCE OVER THE LAST 28 YEARS**

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Background: Aplastic anemia (AA) is a rare, usually acquired disorder characterized by bone marrow failure with bi- or panacytopenia and marrow hypoplasia. The classification into the three main subtypes is of prognostic and therapeutic relevance. Depending on disease severity, patient’s age, and the availability of a potential HLA-identical donor, different therapeutic strategies are favored. Immunosuppressive therapy (IST) with anti-thymocyte globulin (ATG) and cyclosporine (CsA) is considered the initial standard treatment. A hematologic recovery is seen in up to 60-70% of the pts following horse-ATG (hATG) and cyclosporine (CsA) is considered the initial standard treatment. A hematologic recovery is seen in up to 60-70% of the pts following horse-ATG (hATG) and cyclosporine (CsA). PNH clones >10%, the larger clone had to be ≥110% its size. IST was uniform - Cyclosporine (2.5mg/kg p.o. b.i.d.), anti-thymocyte globulin (ATG; ATGAM® 40mg/kg IV daily x 4 days) and (Methylprednisolone 1mg/kg/day x 10 days). CSA doses were adjusted to maintain whole blood trough CSA level of 200-300 μg/L for 12-months followed by slow taper based upon hematologic response. Non-responders at 6 months were eligible to proceed to either a second cycle of ATG or BMT, if a suitable donor was available. Severity of AA [very severe (VSAA), severe (SA) or non-severe (NSA)] and response to IST [(none, partial (PR) or complete (CR)] were determined according to published criteria (Marsh, Br J Haematol, 2009). Statistical comparisons were done using a standard Chi square analysis.

Summary/Conclusions: Flow cytometry for a PNH clone is routinely done in AA although it may be important to evaluate both granulocyte and monocyte clone sizes. Pts with a larger monocyte than granulocyte clone size more frequently have NSA and appear to have a lower response rate to IST. This may have therapeutic implications and could identify a population of pts requiring a unique therapeutic approach.
Background: Stabilizing mutations of NOTCH1 have been identified in about 10% of chronic lymphocytic leukemia (CLL) cases at diagnosis, with a higher frequency in unmutated IGHV-UM (IGHV-UM), immuno-commorosefractory or advanced disease phase CLL, and have been associated with particularly unfavourable prognosis (Rosii et al, Blood, 2012; Del Poeta et al, Br J Haematol, 2013; Stilgenbauer et al, Blood, 2014). In CLL, all NOTCH1 mutations disrupt the C-terminal PEST domain and cause an accumulation of an active NOTCH1 isoform, resulting in a sustained pathway activation.

Aims: To identify molecular/biological features of NOTCH1 mutated CLL.

Methods: The presence of NOTCH1 mutations was investigated by NGS. Gene expression profile (GEP) was performed by a one-color labeling strategy using a one-color labeling strategy using a one-color labeling strategy using a one-color labeling strategy using a one-color labeling strategy.

Results: i) A GEP comparing purified cells of 10 IGHV-UM CLL cases (5 NOTCH1-mut; 15%>37% of NOTCH1 mutated alleles) selected nucleophosmin-1 (NPM1) and ribosomal associated components by western blotting showed a significant difference in NPM1 expression in NOTCH1-wt cases in the IGHV UM subgroup. ii) Western blotting in 11 CLL cases (5 NOTCH1-mut) confirmed a higher NPM1 protein expression in NOTCH1-mut cases, with a direct correlation with NOTCH1 expression (r=0.814). In NOTCH1-mut cases, the NPM1highsubpopulation, isolated by cell sorting, showed a higher NOTCH1 mutational load than the NPM1low subpopulation. iii) IHC treatment of 12 CLL cases (6 NOTCH1-mut) activated NOTCH1 signaling (Rand et al, Mol Cell Biol, 2000), as from HES1 and DTX1 induction, and up-regulated RNA and other RNPs. These results were confirmed by co-culture of CLL cells with the JAGGED1-expressing M2-10B4 stromal cells. Inhibition of NOTCH1 signaling by gamma-secretase-inhibitor L-685,458 or by siRNA for NOTCH1 reduced NPM1 expression (Fig A). iv) Previous studies identified MYC as a direct transcriptional target of NOTCH1 (Rand et al, Mol Cell Biol, 2000) and, in turn, a transcriptional activator for both NPM1 and RNPs. ChIP assays on MEC1-cells, transfected with exogenous NICD, revealed increased NICD binding to the MYC promoter, along with higher expression of MYC, NPM1, and RNPs. Of note, after 48h culture, NOTCH1-mut CLL cases showed increased MYC transcript levels than NOTCH1-wt cases. MYC expression was further increased upon NICD activation by EDTA or by stromal cells co-cultures (Fig B). MYC silencing by siRNA efficiently reduced NPM1 transcript and protein expression. Moreover, CpG-ODN-NIL-2 treatment, to induce MYC overexpression, also increased NPM1 transcript and protein levels in CLL cells. v) NPM1 silencing by siRNA was able to reduce proliferation rates and cell size of both NICO-transfected cells and control cells. In keeping with a NOTCH1-driven regulation of cell growth/proliferation, activation of NOTCH1 signaling in 12 CLL cases (6 NOTCH1-mut) by EDTA or stromal cells co-culture, induced an increase in cell size.

Summary/Conclusions: NOTCH1 mutations in CLL are associated with the overexpression of MYC and MYC-related genes involved in protein biosynthesis including NPM1, which are allegedly responsible for cell growth and/or proliferation advantages of NOTCH1-mut CLL.
NUCLEAR LAMINA REGULATES SOMATIC HYPERMUTATION AND PROGRESSION OF B CELL MALIGNANCIES


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Background: The nuclear periphery, containing the IgH and Igk gene clusters, is a unique compartment comprised of inner nuclear membrane proteins and nuclear lamina. Previous genome-wide and cytological studies revealed the regulatory role for some of these nuclear proteins in higher level genome organisation and gene regulation. In particular, Lamina Associated Domains (LADs) were identified at the nuclear periphery as transcriptionally silent, gene-poor domains, often near the nuclear lamina. More recent studies however revealed an important role of LADs in the regulation of gene expression and recombination. Aims: Given the apparent topological coincidence between LADs and Ig variable clusters, we hypothesised that nuclear lamina might play a paramount role in the dynamics of Ig-encoding variable genome domains. In particular, here we tested whether Lamin B1, a principal LAD-associated component of the nuclear envelope, had any restrictive role on somatic hypermutation (SHM) and the expression of Ig genes. Due to the strong involvement of IgV mutations in the pathogenesis of B-cell malignancies, we also tested whether nuclear lamina is involved in the pathogenesis of germinal centre lymphomas and chronic lymphoid leukaemia (CLL).

Methods: We used BL2 and naïve B cells as in vitro and ex vivo models for somatic hypermutation. ChiP-Seq, ChIP-PCR and ImageStream analyses were performed to establish Lamin B1 genome and nuclear binding dynamics in resting and activated BL2 and B cells. LMB1 RNAi was used to obtain the functional knockdown of Lamin B1 in SHM in vitro. For in vivo studies, OVA immunised mice were used to study Lamin B1 dynamics in the newborn splenic germinal centres. From a translational perspective, pairwise tissue microarray samples of diagnostic and transformed follicular lymphoma were analysed using immunohistochemistry and image analysis. Finally, comparison of statistical analysis of CLL cohort patients was performed to test the impact of LMB1 expression on various clinical parameters in CLL.

Results: We have found that genome binding of Lamin B1, a component of the nuclear envelope involved in epigenetic chromatin regulation, is reduced during B cell activation and formation of lymphoid germinal centres. ChiP-Seq analysis revealed Lamin B1 is located in heavy variable immunoglobulin domains that were released from the Lamin B1 suppressive environment when SHM was induced in B cells. RNAi-mediated reduction of Lamin B1 resulted in spontaneous SHM and the expression of Ig genes. Due to the strong involvement of IgV mutations in the pathogenesis of B-cell malignancies, we also tested whether nuclear lamina is involved in the pathogenesis of germinal centre lymphomas and chronic lymphoid leukaemia (CLL).

Summary/Conclusions: In summary, the results demonstrate that nuclear lamina is involved in the expression of Ig genes and SHM, and that these processes are important for the regulation of germinal centre lymphoma and chronic lymphoid leukaemia.
Background: B cell receptor (BCR) mediated signalling is crucial for the pathogenesis of chronic lymphocytic leukemia (CLL). Drugs such as ibrutinib and idelalisib which inhibit BCR associated kinases have proved effective for the treatment of CLL but only suppress the disease without being curative. Some patients have developed resistance to these drugs following mutations, progress on therapy for unknown reasons, or cannot tolerate these drugs due to adverse events. We have shown that microenvironmental signals (e.g., IL-4) can increase BCR expression and signalling, and can partially reverse the effects of BCR-inhibitor generation. GA1, PTPN22 and FOXP1, GAB1, PTPN22, SOCS1 and SOCS3 assessed by immunoblotting. The effect of cerd on apoptosis was assessed by flow cytometry and PI/Annexin V staining.

Primary human CLL cells treated with IL-4 for 24hr significantly increased expression of positive regulators of BCR signalling FOXP1 and GAB1 in CLL samples with un-mutated IGHV (U-CLL); no change in expression in FOXP1 or GAB1 was seen in CLL samples with mutated IGHV (M-CLL). There was a 40% increase in PTPN22 expression in IL-4-treated U-CLL samples vs no change in M-CLL. Cerd, at therapeutic concentrations, blocked IL-4-mediated increases in FOXP1, GAB1 and PTPN22 and pSTAT6 (a positive control for IL-4 signalling). After 24hr IL-4 selectively increased expression of the negative regulator of IL-4 signalling, SOCS1 and SOCS3 in U-CLL, but not M-CLL cases, and this could be blocked by cerd. Cerd potently inhibited the expression of other cytokines known to play a role in CLL biology (IL-6, IL-10, IL-15, IL-21 and IFN). These results provide evidence that IL-4 may increase BCR signalling in a synergistic manner in the presence of IL-4/CD40L. We now extend these results to assess the important role of this drug combination in the presence of BCR stimulation. The combination of cerd and venetoclax in a synergistic manner in the presence of IL-4/CD40L. We now extend these results to assess the important role of this drug combination in the presence of BCR stimulation. The combination of cerd and venetoclax in the presence of both BCR (bead immobilised anti-IgM) and CD40L, induced synergistic killing, with greater CLL cell death than with either drug alone.

Summary/Conclusions: These results provide evidence that IL-4 may increase BCR signalling by upregulating the expression of positive regulators of BCR signalling in U-CLL and that this can be overcome by cerd. These results support the continued use of cerd in clinical trials for the treatment of CLL, alone or in possible combination with venetoclax.

Aims: To investigate in CLL the influence of VLA-4 expression/activation on ibrutinib response in-vivo.

Methods: VLA-4 expression was assessed by flow cytometry using confirmation sensitive anti-CD29 mAbs (HUTS-21) and LDV-containing VLA-4 ligands, and measured as VLA-4 receptor occupancy (RO) (Chigaev et al. J Biol Chem, 2009). BCR engagement was performed using goat F(ab)2 anti-human IgM. In-vitro studies were carried out on purified VLA-4+ CLL cells exposed in-vivo to ibrutinib. The clinical impact of VLA-4/CD49d expression on ibrutinib treatment was evaluated by measuring the kinetics of absolute lymphocyte count (ALC), the reduction of lymphadenopathy measured as sum of products of the diameters (SPD) % reduction from baseline, and the clinical outcome, as defined by progression free survival (PFS) in CLL patients treated with ibrutinib single agent in the context of name patients program, clinical trials, and real world (n=97).

Results: BCR stimulation (n=27) induced VLA-4 activation (mean RO control vs stimulated: 0.40 vs 0.52, p=0.0006), and increased cell adhesion (stimulated/control: 4.7 vs 7.5; p=0.0002). By comparing day 30 (t30) in vivo ibrutinib-treated CLL cells with pre-treatment (t0), we show that the ibrutinib-dependent BCR signaling impairment, although reducing the constitutive VLA-4 activation (mean RO t0 vs t30: 0.40 vs 0.30, p=0.02) and CLL cell adhesion (mean adhesion t0 vs t30: 4.7 vs 2.1, p=0.013), was overcame by exogenous BCR triggering, which re-activated VLA-4 at levels similar to those of ibrutinib naive cells (mean RO: 0.49 at t30 vs 0.52 at t0). ALC data were available at pre-treatment and at days 30-60-90-120 on ibrutinib in 97 patients (52 CD49d+) (Fig.1A); CD49d+ CLL showed no ALC rise, whereas CD49d- CLL showed the typical ibrutinib-induced ALC peak. After 12 months of continuous treatment, the ALC increased in CD49d- samples but not in CD49d+ CLL. The combination of cerd and venetoclax induced apoptosis in a synergistic manner in the presence of IL-4/CD40L. PFS was inferior in CD49d+ compared to CD49d- CLL (median PFS 39.3 months, vs not reached; p=0.004), even when considering the concomitant presence of TP53 disruption and CD49d+ expression (Fig.1C). A multivariate analysis performed on all patients confirmed the relevance of CD49d+, along with TP53 disruption and UM IGHV mutational status, as independent predictor of shorter PFS in ibrutinib-treated CLL.
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Background: Ibrutinib is an oral Bruton tyrosine kinase (BTK) inhibitor which has advanced the clinical management of CLL. Ibrutinib binds irreversibly to the cysteine 481 residue of the BTK protein, rendering it inactive. BTK inhibition affects the phosphorylation of other intracellular kinases resulting in an immediate redistribution of CLL cells and subsequent apoptosis. We investigated the impact of ibrutinib on the phosphorylation of upstream and downstream kinases in the B-cell receptor pathway in real time in the IcICLLe study (ISRCTN12695534).

Aims: The IcICLLe trial was a single arm, multi-centre feasibility study of ibrutinib in two cohorts of CLL patients: (A) 20 treatment-naïve (TN) requiring treatment according to IWCLL criteria; and (B) 20 relapsed/refractory (RR). All patients received continuous oral therapy with ibrutinib (420mg once daily) from registration until disease progression. The primary endpoint of the trial was the proportion of patients achieving minimal residual disease (MRD) negative remission (depletion of CLL ≤0.01% in peripheral blood (PB) & bone marrow (BM)) within 6 months of trial treatment. Exploratory endpoints included the assessment of phosphorylation of intracellular kinases in the B-cell receptor pathway.

Methods: A panel of markers was assessed on PB & BM taken at screening, and 1 & 6 months. PB was also taken at baseline (0 hours), 4 & 24 hours, 7 & 14 days, and 2, 8 & 16 months. The phosphorylation of Syk, P38α, Btk, pY551, ERK1/2, Akt, S473 was assessed in 4 conditions at each time point: unstimulated +/- ibrutinib, and stimulated with IgM/IgD +/- ibrutinib. 1×10⁶ leukocytes were tagged to extracellular antibodies (CD3/CD19) conjugated to fluorochromes. Ibrutinib (10μM) was added to the cells for 30 minutes at 37ºC followed by anti-IgM/IgD stimulation (10ug/ml). The BD phosflow protocol was followed to lyse/lift/permeate the CLL cells. Antibodies to Btk, pY551, Syk, pY32/3, ERK1/2, Akt, S473 were used tagged to fluorochromes (from BD Biosciences). Cells were acquired on a BD Fortessa flow cytometer.

Results: The phosphorylation of Btk, Syk, Akt and ERK1/2 was analysed in cells at the specified time points and conditions for 20 TN and 20 RR CLL patients. Baseline phosphorylation of all kinases was similar in both PB & BM. Phospho-Btk showed no stimulation on addition of IgM/IgD 4h after initiating therapy. There was a strong (2-4 fold) increase in phosphorylation of Syk kinase with IgM/IgD even in the presence of ibrutinib in vitro. This effect was profound in the first 2 months of ibrutinib therapy with a general decrease in phosphorylation after 6 months. Baseline stimulation of ERK1/2 gave a 1-5 fold increase in phosphorylation but the effect was abrogated within 1 month of ibrutinib therapy. Akt S473 phosphorylation was maintained after 6-12 months of therapy although the degree of phosphorylation decreased at later time points. Syk, Akt and ERK1/2 phosphorylation was unaffected by the addition of ibrutinib in vitro. The pattern of phosphorylation was found to be relatively consistent in responding patients. One patient with progressive CLL had sustained phosphorylation in all markers despite ibrutinib therapy.

Summary/Conclusions: The effect of ibrutinib on the phosphorylation of various kinases in the B-cell receptor pathway was analysed in real time. Syk continued to be phosphorylated over the course of treatment, which is logical as this kinase is upstream of Btk. That the degree of phosphorylation declined over time (even with stimulation) suggests a general inhibitory effect of ibrutinib on CLL cells. ERK1/2 phosphorylation is effectively blocked and there is partial reduction of phosphorylation of Akt S473. Combinations of Btk inhibitor with a Syk or Pd3 kinase inhibitor may result in complete BCR blockade. Phosphorylation patterns may also act as an adjunct to ascertain the response to therapy.

EVALUATION OF COMBINATIONAL THERAPIES FOR RELAPSED/REFRACTORY CLL WITH MUTATED P53

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Background: Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with survival ranging from months to decades. CLL patients harboring TP53 alterations are well known to be refractory to standard therapies; however, recent studies indicate that ibrutinib, a Bruton’s tyrosine kinase (BTK) inhibitor, suppresses the B-cell receptor (BCR) signaling pathway and is an effective treatment option for these patients. Unfortunately, many patients with TP53 alterations will ultimately fail ibrutinib-based therapies. Similarly, we have used a mouse model of refractory p53 mutant CLL (Eμ-TCL1;p53R172H), and reported that while ibrutinib is effective in reducing the CD5+CD19+ population and extending survival, these mice eventually succumb to the disease (Lee HJ, BJC 2016). These incomplete therapeutic responses indicate that ibrutinib provides only a temporary respite for this refractory disease, and highlights our need to develop more potent and targeted combinations.

Aims: Ibrutinib is effective in delaying (but not eliminating) leukemic progression in p53 mutant CLL, suggesting that combinational therapies that inhibit BCR signaling and activate apoptotic programs may be effective therapeutic strategies. Thus, agents that do not require activation of p53 but are effective in blocking oncogenic pathways (BTK and BCL-2) are attractive options. Currently, ibrutinib and ABT-199 meet this criteria and thus, we hypothesize that simultaneous inhibition of the BTK- and BCL-2-pathways will be an effective strategy in treating p53 mutated CLL.

Methods: To test this, we used RNA-Seq to examine expression changes in B-cells from Eμ-TCL1 mice carrying either wild type or a single p53R172H hot-spot mutation (corresponding to p53R172H in humans) following ibrutinib treatment. qRT-PCR and IHC were used to validate expression of key targets within pathways amenable to combinational therapy. Hematopoietic tissues were subjected to combinational therapies to interrogate efficacy.

Results: We have shown that ibrutinib downregulates the BTK- and ERK-pathways regardless of p53 status. However, less is known in regards to global expression changes in p53 mutant CLL following BTK inhibition. To investigate this, we performed RNA-Seq analyses using malignant B-cells from untreated and ibrutinib treated Eμ-TCL1;p53R172H and Eμ-TCL1 mice. Pathway analyses revealed that CLL cells harboring a single p53 mutant allele retained a partial ability to activate p53-dependent programs. qRT-PCR revealed robust activation of p53-dependent anti-proliferative targets like p21, but only modest activation of pro-apoptotic targets (e.g., PUMA), suggesting these p53 mutant CLL cells possess diminished capacity to activate apoptosis or overcome apoptotic inhibitors. To explore this altered bi-modal p53 activation, we performed IHC and observed that apoptotic activation was hampered by increased BCL-2 expression. To examine whether this BCL-2-dependent inhibition could be overcome, malignant B-cells were treated with ibrutinib alone, ABT-199 (a BCL-2 inhibitor) alone, or in combination. Here, we observed that ABT-199 was sufficient to activate apoptosis, regardless of p53 status, and that its use in combination with ibrutinib drastically reduced cell viability.

Summary/Conclusions: Together, these data indicate that patients with a partially attenuated p53 pathway may retain the ability to activate apoptosis if molecular barriers are removed (e.g., BCL-2 via ABT-199). Furthermore, these results suggest that combinations with BTK- and BCL-2 inhibitors may be therapeutically beneficial for patients with mutated TP53.
Chronic myeloid leukemia - Biology

P591
THE DNA REPLICA TION PATHWAY HAS POTENTIAL PREDICTIVE VALUE FOR TKI RESPONSE AND THERAPEUTIC INTERVENTION IN CHRONIC MYELOID LEUKAEMIA
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Background: Chronic myeloid leukemia (CML) is a myeloproliferative disorder which arises in a haematopoietic stem or multipotent progenitor cell with the t(9;22)(q34;q11) chromosomal translocation. Tyrosine kinase inhibitors (TKIs) were developed to target the constitutively active oncoprotein BCR-ABL, which is expressed as a result of this translocation. TKI therapy has significantly improved patient survival, however predicting response to therapy is one of the unmet clinical challenges in CML. Moreover, TKIs are unable to target the leukemic stem cells (LSCs) which drive the disease; persistence of the LSC therefore remains a major obstacle to curing CML. Understanding the mechanisms that LSC employ to survive TKI treatment is necessary to design essential therapeutic strategies to eliminate CML in the future.

Aims: To identify genes with predictive value for TKI response and to determine the efficacy of drug targeting one of the key pathways identified.

Methods: Microarray, Fluidigm, Real-time PCR, FACS based cell cycle and Annexin V apoptosis analysis, Trypan blue exclusion cell counts.

Results: Analysis of bulk CML patient microarray data (GSE 47927) identified 323 deregulated genes either in the stem cell population or during disease progression. DNA damage response genes are important for self-renewal, DNA damage repair, cell cycle and survival. These genes were validated in 60 samples from the SPIRIT 2 clinical trial [a multicentre phase III randomised trial comparing the TKI imatinib (400mg daily) versus Dasatinib (100mg Daily)] with 18 months follow-up data regarding molecular response to TKI treatment. Patients were stratified as good/intermediate/poor responders to TKI and the gene signature significantly differentially expressed was identified. These data highlighted the DNA repair genes as having potential predictive value, in particular, the monochromosome maintenance (MCM) protein and origin of replication (ORC) family of genes, involved in DNA replication and cell cycle regulation. Single cell analysis of CD34+ cells across the patient cohort identified considerable heterogeneity of expression of MCMs and ORCs, with ORC3, in particular, exhibiting a different expression profile in good/intermediate/poor responders (n=3 of each). In addition single cell analysis highlighted a significant difference in the expression of MCM2, -4, -7 & ORC2 in the most primitive LSC (CD34+CD38−Lin−) compared to CD34+CD38−Lin+ cells. Next, we investigated the ability of telomere shortening (HTS), a potent helicase inhibitor of MCM on its own and in combination with IM to target the CML cell line K562. Our extensive dose and time response studies followed by FACS-based apoptosis and cell cycle analysis proved the potency of HTS and its synergistic action in combination with imatinib. We also investigated the changes in MCM and ORC family in cell cycle and DNA damage response genes at the transcript level in response to HTS and imatinib in the K562 cell line. Overall the data generated indicates that targeting the MCM pathway in combination with BCR-ABL inhibition is a rational approach for future therapeutic intervention in CML.

Summary/Conclusions: Global ‘omics’ experimental approaches are valuable for identifying novel pathways deregulated in CML. This combined with single cell ‘omics’ studies enable the heterogeneity of gene expression and the response of individual LSCs to TKI to be evaluated. Our data indicate that the DNA replication pathway plays an important role in CML, with levels of MCMs and ORCs having potential predictive value in TKI response and are a promising drug target in CML.

P592
SIGNAL TRANSDUCING ADAPTOR PROTEIN-1 (STAP-1) MAINTAINS CHRONIC MYELOID LEUKEMIC STEM CELLS
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Background: Signal transducing adaptor protein (STAP) -2 was cloned as a c-fms binding protein. Previously, we have demonstrated that STAP-2 binds to BCR-ABL, which is constitutively activated in chronic myeloid leukemia (CML), via its SH2-like domain and enhances BCR-ABL activity leading to activation of downstream molecules. Global ERK, STATs, BCL-xL and BCL-2. The family of STAPs includes STAP-1, identified as a c-kit interacting protein, and STAP-2. While STAP-2 is expressed ubiquitously, STAP-1 has hematopoietic-specific expression in mice. It is still unknown whether STAP-1 plays a role in CML, although STAP-1 is expected to have similar functions based on the structural homology between STAP-1 and STAP-2.

Aims: To elucidate the role of STAP-1 in CML using mouse model and human samples.

Methods: We generated STAP-1 deficient mice of the C57BL/6J genetic background. For establishment of CML mouse model, we isolated Lineage (Lin)−Sca-1+ c-kithigh (LSK) fraction of bone marrow (BM) cells from STAP-1+/+ and STAP-1−/− mice, infected them with retrovirus carrying MSCV-BCR-ABL-ires-GFP, and transplanted into congenic recipients, that were named Wild type (WT) and STAP-1−/−CML mice, respectively. Human BM samples were collected after informed consent, using protocols approved by the Investigational Review Board of Osaka University Hospital.

Results: Using Western blot and immunoprecipitation assay, we confirmed that STAP-1 binds to BCR-ABL. CML mouse model was then employed to analyze the role of STAP-1. We found that STAP-1−/− CML mice showed significantly longer survival than WT CML mice (Fig. 1). STAP-1−/− CML mice displayed less splenomegaly and lung hemorrhages compared to WT, suggesting that loss of STAP-1 attenuates CML progression. To investigate how STAP-1 regulates CML progression, we evaluated leukemic stem cells (LSCs) in CML mice. The absolute numbers of STAP-1−/− LSCs (GFP+ LSK) in BM and spleen were significantly lower than those of control (WT vs STAP-1−/−; 2090.3 ± 694.07 cells vs 412.57 ± 114.07 cells in BM, p=0.0291; 12.9 ± 1.75 x104 cells vs 4.09 ± 0.72 x104 cells in Spleen, p=0.0009). In colony-forming assay in vitro, STAP-1−/− LSCs generated less colonies in the first and second plating compared to WT LSCs. These data indicated that deletion of STAP-1 would impair self-renewal capacity of LSCs. When we transplanted STAP-1−/− or STAP-1+/+ mice without BCR-ABL transduction in the presence of competing BM cells, deletion of STAP-1 had no effects on engraftment at 28 days after transplantation. Furthermore, we measured the expression of STAP-1 in BM cells derived from patients in the chronic phase of CML. As a result, STAP-1 mRNA was abundant in the LSC (CD34+CD38−Lin−) compartment.

Figure 1.

Summary/Conclusions: In this study, we utilized CML mouse model and showed that STAP-1 is required for progression of CML. Our findings indicate that STAP-1 has an indispensable role in LSC maintenance, while normal hematopoietic stem/progenitor cells were not affected by STAP-1 deficiency. Although a majority of patients have a durable response to BCR-ABL tyrosine kinase inhibitors, the outcome of patients who fail the treatment due to primary or acquired resistance is still miserable. Our findings in mice and human suggest that STAP-1-1 could be a suitable drug target for CML. Further analysis will be needed to clarify the molecular mechanisms by which STAP-1 regulates the progression of CML and maintains survival of LSCs.

P593
TELOMERE SHORTENING IN CD34+38- BCR-ABL POSITIVE BONE MARROW CELLS FROM NEWLY DIAGNOSED PATIENTS WITH CML CORRELATES WITH THE CLONE SIZE OF THE LEUKEMIC STEM CELL COMPARTMENT
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Background: Chronic myeloid leukemia (CML) is a clonal stem cell disorder characterized by the BCR-ABL translocation. Previous work provides evidence that based on the size of the leukemic stem cell (LSC) clone within the CD34+38− population at diagnosis, chronic phase (CP) of CML can be stratified into early and late CP. Patients in late CP have a higher LSC burden going along with an inferior response to TKI therapy. Telomeres shorten with each

haematologica | 2017; 102(s2) | 231

Madrid, Spain, June 22 – 25, 2017
cell division and telomere length (TL) in peripheral blood cells has been shown to correlate with disease stage, response to treatment and duration of CP in CML patients. However, the use of TL as a routine clinical biomarker in CML has been complicated by considerable inter-individual, mostly genetic variability in TL ideally requiring non-clonal control cells.

**Aims:** Based on these considerations, we used a modified Q-FISH technique in a large clinical study to test the hypothesis that BCR-ABL+ LSCs or BCR-ABL- control cells within the CD34+38- hematopoietic stem cell compartment of diagnostic patients with CML in CP.

**Methods:** 15 patients (median age: 59 years, range: 41-72 years) diagnosed with CML in CP of the NCT00852566 study (Nordic CML Study Group) were retrospectively analyzed. For the patients with successful cytogenetic follow-up, this study was focused on 41 patients. Of those, 2 (14%) belonged to the Sokal high risk group, 5 (36%) to intermediate and 7 (50%) to the low risk group. CD34+38- cells sorted from bone marrow samples were tested with the standard FISH method using dual fusion dual color BCR-ABL FISH probes following standard procedures. After capturing the BCR-ABL staining using confocal microscopy, samples were re-processed for TL analysis by Q-FISH using established protocols. TL staining was analyzed in all previously captured cells allowing the identification of BCR-ABL+/-cells within the same sample. Analysis and quantification of BCR-ABL FISH staining and TL measurement by Q-FISH were performed in blinded fashion.

**Results:** Overall, we observed significantly shortened TL in the BCR-ABL+ compared to BCR-ABL- cells (-4.9 arbitrary units (a.u.)) range: 3.7 to 16.9 a.u., p=0.04). Next, we correlated the clone size (i.e. the proportion of BCR-ABL+ positive cells within the CD34+38- compartment) with the degree of telomere shortening in LSC. Mean clone size of the patients was 59.9 ± 22.0 % S.D. Of note, we found a significant negative correlation (R²=0.36, p=0.02) between TL and clone size strongly supporting the notion that increased expansion of the BCR-ABL+ LSC pool leads to accelerated telomere shortening. Comparing the median TL measured by Q-FISH (R²=0.28, p=0.04) of BCR-ABL+ cells to the Sokal (R²=0.04, p=0.38) score did not reveal any statistically significant correlation with the degree of telomere shortening probably due to the small sample size analyzed in this pilot study.

**Summary/Conclusions:** In this study, we provide further evidence for accelerated telomere shortening in BCR-ABL+ LSC as compared to their normal CD34+CD38- counterpart in CP CML samples at diagnosis. Interestingly, the degree of TL shortening linearly correlates with the clone size of the BCR-ABL+ LSC compartment. Thus, this retrospective study (now on the LSC level) further supports a role of TL as a prognostic and predictive biomarker in newly diagnosed patients with CML pending confirmation in prospective trials.

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**P594**

**GENOMIC CHARACTERIZATION OF CML AT DIAGNOSIS REVEALS PREEXISTING SOMATIC MUTATIONS THAT MAY PREDICT PROGRESSION TO BLASTIC PHASE INDEPENDENTLY OF BCR-ABL1 MUTATIONS**

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**Background:** Blastic phase of chronic myeloid leukemia (BP-CML) remains mostly incurable even with newer generation tyrosine kinase inhibitors (TKI) and represents an unmet clinical need. Although in recent years a dramatic reduction in the transformation of chronic phase (CP-CML) to BP-CML has been observed, still up to 5% of patients will progress to BP-CML despite treatment with TKI. Prospective identification of such patients may have a significant clinical impact.

**Aims:** To analyze the spectrum of somatic mutations in two groups of CML patients with clinically different disease course: first group (BP) comprised of 11 patients who progressed to BP-CML despite treatment with TKI and/or allo-HSCT (one patient) and died (paired samples from Dx and BP were analyzed); second group (MMR) included Dx samples from 36 patients who achieved major molecular response (MMR) on TKI within 6 months and remained in remission. The second group (MMR) included Dx samples from 36 patients who achieved major molecular response (MMR) on TKI within 6 months and remained in remission; the subsequent analysis was focused on putative protein damaging variants, supported by variant effect prediction tools such as PolyPhen2, SIFT or CHASM. All reported variants were reconfirmed by Sanger sequencing.

**Results:** The BP group comprised of paired samples from 11 CML patients who progressed to BP and died despite treatment with TKI. Median age at diagnosis was 53y (range 26-77), median time to progression for 9 patients (2 were diagnosed in accelerated phase or BP) was 17.5 months (mo) (range 4-108) and median survival was 22 mo (range 10-116). None of those patients harbored BCR-ABL1 mutation at the time of Dx and progression to BP-CML, 4 patients had additional chromosomal alterations at progression to BP including two frequent (trisomy 8 and monosomy 7). Targeted enrichment followed by NGS allowed us to achieve deep coverage (>80% ge50). Median number of rare variants was 26 (range 18-38) and 29 (range 23-32) for Dxs and progression samples respectively. In the MMR group, 9 patients were reprocessed for TL analysis by Q-FISH using established protocols. TL staining was analyzed in all previously captured cells allowing the identification of BCR-ABL+/-cells within the same sample. Analysis and quantification of BCR-ABL FISH staining and TL measurement by Q-FISH were performed in blinded fashion.

**Results:** Overall, we observed significantly shortened TL in the BCR-ABL+ compared to BCR-ABL- cells (-4.9 arbitrary units (a.u.)) range: 3.7 to 16.9 a.u., p=0.04). Next, we correlated the clone size (i.e. the proportion of BCR-ABL+ positive cells within the CD34+38- compartment) with the degree of telomere shortening in LSC. Mean clone size of the patients was 59.9 ± 22.0 % S.D. Of note, we found a significant negative correlation (R²=0.36, p=0.02) between TL and clone size strongly supporting the notion that increased expansion of the BCR-ABL+ LSC pool leads to accelerated telomere shortening. Comparing the median TL measured by Q-FISH (R²=0.28, p=0.04) of BCR-ABL+ cells to the Sokal (R²=0.04, p=0.38) score did not reveal any statistically significant correlation with the degree of telomere shortening probably due to the small sample size analyzed in this pilot study.

**Summary/Conclusions:** In this study, we provide further evidence for accelerated telomere shortening in BCR-ABL+ LSC as compared to their normal CD34+38- counterpart in CP CML samples at diagnosis. Interestingly, the degree of TL shortening linearly correlates with the clone size of the BCR-ABL+ LSC compartment. Thus, this retrospective study (now on the LSC level) further supports a role of TL as a prognostic and predictive biomarker in newly diagnosed patients with CML pending confirmation in prospective trials.

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**P595**

**INCREASED INDOLEAMINE 2,3-DIOXYGENASE (IDO1) ACTIVITY IN EARLY CHRONIC PHASE CHRONIC MYELOGENOUS LEUKEMIA (CML-CP) IS REDUCED BY NILOTINIB THERAPY AND PREDICTS MOLECULAR RESPONSE**

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**Background:** Indoleamine 2,3 dioxygenase (IDO1) is the rate-limiting enzyme in the metabolism of the essential amino acid tryptophan (TRP). IDO1 is induced mainly by interferons during infection and inflammation. Strong IDO1 activity depletes tryptophan, which results in reduced T cell activation and proliferation as well as expansion of immunosuppressive regulatory T cells. Deregulation of IDO1 activity has been linked to cancer immune evasion, but its role in chronic phase (CP) CML has not been investigated in detail.

**Aims:** Determination of IDO1 levels and activity in plasma CML-CP patients in the course of tyrosine kinase inhibitor therapy and their correlation with clinical and immunological parameters as well as molecular response.

**Materials and Methods:** On a large cohort of 227 consecutive CML patients of the IDO-pathway (soluble IDO1=sIDO1 and kynurenine/tryptophan ratio=kYN/TRP) as a product of IDO1 activity) as well as various leukocyte populations such as plasmacytoid dendritic cells (pDC) were analyzed alongside the prospective pan-European ENEST1st clinical study (NCT01611777). This study included 50 patients who received chemotherapy (identical CP-CML) or were subsequently treated with 300mg BID imatinib and longitudinally analyzed at months 6 and 12 of therapy. Molecular responses were quantified in central EUTOS reference laboratories.

**Results:** The soluble IDO (sIDO1) levels and kYN/TRP ratio are significantly up-regulated in newly diagnosed CP-CML and drop during nilotinib therapy. sIDO1 levels significantly correlate with increased kYN/TRP, suggesting increased IDO1 activity at diagnosis. Increased sIDO1 is linked to a pro-inflammatory status in CML patients, as it positively correlates with increased serum neopterin levels as well as to various other pro-inflammatory markers, such as IFN-g, IL-6, IL-10 and TNFα. soluble IDO1 and kYN/TRP correlated negatively with the proportion of pDC, the main producers of IFN-c. Interestingly, a higher KYN/TRP is linked to superior molecular response, as demonstrated by a significant correlation...
of the KYN/TRP ratio to BCR-ABL transcript levels. Patients having a high KYN/TRP ratio (> mean +2SD of post therapy levels) reach deep molecular response rates (i.e. MR≥5) significantly earlier and at higher rates. Moreover, combining KYN/TRP with sCD62L levels, a recently identified predictive biomarker, resulted in a score robustly predicting the odds of achieving deep molecular response.

Summary/Conclusions: CML diagnosis in CP is linked to an increased inflammatory status, as shown by increased levels of sIDO and its metabolites kynurenine leading to an increased KYN/TRP ratio. In solid cancer increased IDO expression/activity is linked to inferior outcome by favoring immune evasion. In contrast, in CML an increased KYN/TRP ratio is associated with improved outcomes. Therefore, to implement testing for IDO in the routine diagnostic surveillance to provide a basis of optimized clinical management of patients treated with ponatinib.

Background: Despite the dramatic improvement of prognosis in CML patients due to the introduction of tyrosine kinase inhibitors (TKIs), resistance occurs in a considerable proportion of patients. The best-characterized mechanism of resistance is the acquisition of mutations in the BCR-ABL1 tyrosine kinase domain (TKD) affecting TKI binding. The third-generation TKI ponatinib exerts strong anti-neoplastic effects even in advanced CML stages and is capable of suppressing the kinase activity of BCR-ABL1 carrying any single mutation including T315I. Nevertheless, resistance to ponatinib can evolve in sub-clones carrying BCR-ABL1 variants with two or more mutations on the same allele, if the IC50 values for this TKI exceed the maximum achievable effective plasma levels (effcmax). These co-called compound mutations (CMs) are associated with increased oncogenic potential in comparison to individual mutations, and represent a powerful mechanism of potential resistance to all currently available TKIs. The occurrence of compound mutations has been linked particularly to sequential treatment with different TKIs, and the identification of their responsiveness to ponatinib is of paramount importance for the subsequent clinical management.

Aims: 1. To determine the spectrum of highly TKI-resistant CMs. 2. Measure the responses of BCR-ABL1 CMs to ponatinib

Methods: We have established a BCR-ABL1 CM protein model facilitating assessment of the presumptive impact of 27 different CMs involving important functional sites of the BCR-ABL1 TKD, and including constellation expected to display high resistance to ponatinib. To assess the anticipated responses to ponatinib in vitro, we have introduced all BCR-ABL1 CMs into Ba/F3 cells using a recently published transposon-mediated approach (Byrgazov et al., Oncotarget 2016, 7(47):78083-78094), and IC50 values of these CMs were derived of

Results: Most CMs involving sites with no previous evidence for implication in resistance to ponatinib displayed IC50 values below 10 nM. This effcmax is readily achievable even with the 15mg daily dose of ponatinib. CMs revealing elevated resistance to ponatinib in vitro almost invariably included T315I or F317L mutations. While most CMs involving T315I revealed very high IC50 values, some of the predicted compound mutations containing F317L displayed an IC50 for ponatinib in the range of the effcmax achievable only with a daily dose of 45mg. These observations are supported by clinical findings in the PACE trial which revealed impaired responses of patients with CMs involving F317L who had received average daily doses of ponatinib below 45mg (Deininger et al., Blood 2016, 127(6):703-12).

Summary/Conclusions: Current strategies that aim at decreasing the dose of ponatinib to prevent severe side effects should carefully consider the presence and type of mutations in the BCR-ABL1 TKD to enable effective treatment. It would therefore be of benefit, to implement testing of the above relevant drug concentrations and monitoring the kinetics of mutant subclones covering also compound mutations in the routine diagnostic surveillance to provide a basis for optimized clinical management of patients treated with ponatinib.

P597

IS THERE EFFECTIVE IMMUNE SURVEILLANCE AGAINST CHRONIC MYELOID LEUKAEMIA? NO

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Background: Immune surveillance refers to a process whereby the innate and adoptive immune systems recognize and eliminate cancer cells on the basis of cancer-specific or related antigens or other molecules. Substantial data support the concept of immune surveillance in rodents but data in humans are less convincing and apply mostly to virus-induced cancers. However, considerable data indicate a strong anti-leukaemia effect in persons with chronic myeloid leukaemia (CML) receiving an allogeneic haematopoietic cell transplant. Most, if not all, of this anti-leukaemia effect is conferred with graft-versus-host disease (GvHD) and whether there is a allogeneic or host specific anti-leukaemia effect distinct from GvHD is controversial. Another interesting observation is about 40 percent of persons with CML and a deep molecular response to tyrosine kinase-inhibitors (TKIs) remain in molecular remission when TKI-therapy is stopped. Because TKI-therapy is very unlikely to eradicate every CML cell, some attribute this therapy-free remission (TFR) to a result of immune surveillance. Immune surveillance refers to a process whereby the innate and adoptive immune systems recognize and eliminate cancer cells on the basis of cancer-specific or related antigens or other molecules. Substantial data support the concept of immune surveillance in rodents but data in humans are less convincing and apply mostly to virus-induced cancers. However, considerable data indicate a strong anti-leukaemia effect in persons with chronic myeloid leukaemia (CML) receiving an allogeneic haematopoietic cell transplant. Most, if not all, of this anti-leukaemia effect is conferred with graft-versus-host disease (GvHD) and whether there is a allogeneic or host specific anti-leukaemia effect distinct from GvHD is controversial. Another interesting observation is about 40 percent of persons with CML and a deep molecular response to tyrosine kinase-inhibitors (TKIs) remain in molecular remission when TKI-therapy is stopped. Because TKI-therapy is very unlikely to eradicate every CML cell, some attribute this therapy-free remission (TFR) to a result of immune surveillance. Immune surveillance refers to a process whereby the innate and adoptive immune systems recognize and eliminate cancer cells on the basis of cancer-specific or related antigens or other molecules. Substantial data support the concept of immune surveillance in rodents but data in humans are less convincing and apply mostly to virus-induced cancers. However, considerable data indicate a strong anti-leukaemia effect in persons with chronic myeloid leukaemia (CML) receiving an allogeneic haematopoietic cell transplant. Most, if not all, of this anti-leukaemia effect is conferred with graft-versus-host disease (GvHD) and whether there is a allogeneic or host specific anti-leukaemia effect distinct from GvHD is controversial. Another interesting observation is about 40 percent of persons with CML and a deep molecular response to tyrosine kinase-inhibitors (TKIs) remain in molecular remission when TKI-therapy is stopped. Because TKI-therapy is very unlikely to eradicate every CML cell, some attribute this therapy-free remission (TFR) to a result of immune surveillance. Immune surveillance refers to a process whereby the innate and adoptive immune systems recognize and eliminate cancer cells on the basis of cancer-specific or related antigens or other molecules. Substantial data support the concept of immune surveillance in rodents but data in humans are less convincing and apply mostly to virus-induced cancers. However, considerable data indicate a strong anti-leukaemia effect in persons with chronic myeloid leukaemia (CML) receiving an allogeneic haematopoietic cell transplant. Most, if not all, of this anti-leukaemia effect is conferred with graft-versus-host disease (GvHD) and whether there is a allogeneic or host specific anti-leukaemia effect distinct from GvHD is controversial. Another interesting observation is about 40 percent of persons with CML and a deep molecular response to tyrosine kinase-inhibitors (TKIs) remain in molecular remission when TKI-therapy is stopped. Because TKI-therapy is very unlikely to eradicate every CML cell, some attribute this therapy-free remission (TFR) to a result of immune surveillance.
Methods: To test these hypotheses, we studied whether there was an increased incidence in CML in persons receiving immune suppression, after solid organ transplants. IF immune surveillance is important in CML we would expect an increased incidence in this setting. We used a dataset from the Collaborative Transplant Study (CTS) which collects information on recipients of solid organ transplants beginning in 1985 from >300 transplant centers worldwide. Cancer incidence data were checked annually by questionnaire. Data for expected CML incidence were obtained from a cohort of identical size matched for age and sex from Cancer Incidence in Five Continents monitored for the same duration as the transplant cohort. Data collection and processing were approved by the Data Protection Agency in Germany and all participating centers are in agreement with local ethical and privacy regulations.

The CTS dataset consisted of 441,332 recipients of kidney (N=355,606), liver (N=47,846) and heart (N=37,880) transplants. Amongst kidney transplant recipients the standardized incidence ratio (SIR) for developing CML was 1.54 (95% confidence interval, 1.1, 2.1; p<0.01) representing 39 cases in 1,682,491 person-years at-risk (at-risk cases). Amongst liver transplant recipients the SIR was 1.72 (0.6, 4.0; P=0.34) representing 5 cases in 182,833 person-years at-risk vs. 3 expected (2 excess cases). Amongst heart transplant recipients the SIR was 3.47 (1.8, 6.1; p=0.0005) representing 12 cases in 173,015 person-years at-risk vs. 3 expected (9 excess cases). Data from recipients of kidney and liver transplants suggest immune suppression does not increase risk of developing CML or does so very slightly. The increase in SIR in kidney graft recipients is generally-attributed to increased cancer surveillance including blood testing. Although the SIR of CML was substantially-increased after heart transplants, these persons receive high doses of ionizing radiations for diagnostic and therapeutic procedures such as computer tomography (CT)-angiography. Ionizing radiations are a proved cause of CML which might explain the increased SIR.

Results: Our data, 25 excess cases of CML in 2,038,339 person-years at-risk observation suggest the magnitude of immune-surveillance do not support the hypothesis that this immune surveillance operates to an important extent to prevent CML in humans.

Summary/Conclusions: Consequently, the anti-leukaemia effect associated with allotransplants and the TFR observed after stopping TKI-therapy is unlikely to result from effective immune surveillance against CML.

Background: In newly-diagnosed chronic phase (CP)-CML patients, 15–30% who start first-line tyrosine kinase inhibitors (TKIs) therapy will not reach an optimal response, and a BCR-ABL1 kinase domain (KD) mutation will be detectable in 25–50% of patients with treatment failure with an increased frequency of these mutations observed in accelerated phase and blast crisis patients. Currently, Sanger sequencing (SS) technique analyzing BCR-ABL1 is considered the gold standard for mutation detection knowing that this assay has a sensitivity of around 20 %, and therefore is unsuitable for identifying low-level variants (<20 % variant frequency). Recently next generation sequencing (NGS)-based assays have been reported for detecting BCR-ABL1 KD mutations; although these NGS strategies are more accurate and precise than SS, their level variants (<20 % variant frequency). Recently next generation sequencing (NGS)-based assays have been reported for detecting BCR-ABL1 KD mutations; although these NGS strategies are more accurate and precise than SS, they are burdened by costs related to the initial investment, that is the sequencer purchase, the preparation of specific targets libraries, and the required reagents. MiONiON is a single molecule sequencer connected to a laptop through a USB3.0 interface, based on nanopore technology; it works by connecting two strands of DNA molecules by a hairpin, and sequencing them consecutively.

Aim: To describe a third-generation sequencing assay on MinION for detecting BCR-ABL1 KD mutations and compare the results to a SS-based test in 24 Ph+ leukemia cases.

Methods: Overall, 24 patients were included; among them, 12 (11 CML and 1 ALL cases) developed treatment resistance during the TKI’s treatment course (Group 1) and 12 (7 CML and 5 ALL) cases were at diagnosis (Group 2). For the second run included the Group 2 and lasted 24 hours to achieve a deeper sequencing and negative samples (K Cohen=0.690; p <0.02): 77 samples were concordant (33.4%) in MR5.0. On the other hand, of the 19 negative samples with the lower sensitivity of around 20 %, and therefore is unsuitable for identifying low-level variants (<20 % variant frequency). Recently next generation sequencing (NGS)-based assays have been reported for detecting BCR-ABL1 KD mutations; although these NGS strategies are more accurate and precise than SS, they are burdened by costs related to the initial investment, that is the sequencer purchase, the preparation of specific targets libraries, and the required reagents. MiONiON is a single molecule sequencer connected to a laptop through a USB3.0 interface, based on nanopore technology; it works by connecting two strands of DNA molecules by a hairpin, and sequencing them consecutively.

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ROLE OF THE AURORA KINASE A/PLK1 AXIS INHIBITION IN RESTORATION OF CELL GROWTH CONTROL OF CHRONIC MYELOID LEUKEMIA PROGENITORS

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Summary/Conclusions: In a large series of patients the automated and manual molecular methods, applied in 4 different laboratories, resulted comparable in classification of patients in “molecular classes”. The advantage of the “Ultra technique is represented by the higher number of detected ABL1 copies and the easier standardization.

P600

Chronic myeloid leukemia - Clinical 2

P601

DURABLE TREATMENT-FREE REMISSION (TFR) FOLLOWING FRONTLINE NILOTINIB (NIL) IN PATIENTS (PTS) WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP): ENSFREEDOM 96-WK UPDATE

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Background: EN SFREEDOM (NCT017184068) is evaluating the ability to stop NIL and to achieve a TFR lasting >48 wk in pts with a sustained deep molecular response (MR4.5) on frontline NIL. Previous results from EN SFREEDOM showed that 51.6% of pts (98/190) who attempted TFR remained off treatment and in major MR (MMR; BCR-ABL1 ≤ 0.1% on the International Scale) at 48 wk.

Aims: To analyze updated TFR data and predictive factors for remaining in TFR in EN SFREEDOM.

Methods: Eligible pts had CML-CP with b2a2 and/or b3a2 BCR-ABL1 transcripts, ≥2 y of frontline NIL, and MR4.5 (BCR-ABL1 ≤ 0.0032%) prior to enrollment. All pts provided informed consent. After enrollment, pts continued NIL for 1 y (consolidation phase). MR was assessed every 12 wk during the 1-y consolidation phase; pts with no assessment worse than MR4 were grouped according to Sokal risk score at diagnosis or depth of response prior to attempting TFR (based on response assessments in the consolidation phase), and 48-wk TFR rates in each subset were calculated. The current analysis was conducted when all pts who entered TFR had completed 96 wk of TFR, reintilated NIL, or discontinued from the study (data cutoff, 31 Oct 2016).

Results: Of 190 pts who entered TFR, 93 (48.9% [95% CI, 41.6% - 56.3%]) remained in MMR and off treatment at wk 96, including 88 (46.3%) who were in MR4.5. Three pts who were in TFR at 48 wk lost MMR by wk 96, and 2 additional pts discontinued from the study between 48 and 96 wk without losing MMR. Among pts with low, intermediate, or high Sokal risk at diagnosis, 39/62 (62.9% [95% CI, 49.7% - 74.8%]), 25/50 (50.0% [95% CI, 35.5% - 64.5%]), and 9/28 (32.1% [95% CI, 15.9% - 52.4%]), respectively, remained in TFR at wk 49 (Sokal risk scores were missing for 50 pts). Among pts who entered TFR for ≥2 y in all risk groups according to Sokal risk score at diagnosis or depth of response, 88/88 (100.0%) remained in TFR at wk 48 vs 82/80 (40.0% [95% CI, 19.1% - 63.9%]) who had ≥1 assessment between MR4 and MR4.5 during the consolidation phase. Overall, of 88 pts who reintilated NIL due to loss of MMR, 87 (98.9%) regained MMR and the remaining pt left the study 7.1 wk after NIL reinitiation without regaining MMR; 81 of 88 pts (92.0%) regained MR4.5 by the data cutoff. Among pts remaining in TFR for >48 wk (n=100), adverse events (AEs) were less frequent during the second vs the first 48 wk of TFR, 2 (2.0%) and 1 (1.0%), respectively. Three pts had cardiovascular AEs during the study: 2 second 48 wk of TFR, respectively; 34 (34.0%) and 9 (9.0%), respectively, had AEs in the predefined musculoskeletal pain grouping.

Summary/Conclusions: The majority of pts in TFR at 48 wk remained in TFR at 96 wk, and they reported fewer AEs during the second 48 wk of TFR than in the first 48 wk, affirming the durability and safety of TFR following NIL. No strong predictive factors for remaining in TFR were identified. Pts with low Sokal risk and pts with continuous MR4.5 in the consolidation phase tended to have higher TFR rates than other pts, although these results must be interpreted with caution due to the small number of pts in some subsets and the wide 95% CIs. Additionally, the biological explanation for an association between Sokal risk score at diagnosis and a subsequent ability to remain in TFR is unknown. These results support TFR as a valuable option for pts in sustained DMR on frontline NIL.

P602

RESPONSE DIFFERENCES IN THE BCR-ABL1 E13A2 AND E14A2 VARIANTS MAY BE A TECHNICAL QPCR ARTIFACT

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Table 1.

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<td><strong>Conclusions:</strong> The advantage of using AK and Plk1 inhibitors in CML therapy mostly results from effects independent from TK activity of Bcr-Abl protein. We proved that the AK and Plk1 inhibitors induce growth arrest and apoptosis in IM sensitive and resistant cell lines.</td>
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Background: The t(9;22) translocation in chronic myeloid leukemia (CML) generally occurs in intron 12 or 13 of the BCR gene resulting in two different transcripts, the e13a2 or e14a2. It has been suggested that the two variants represent separate disease entities and that the transcript variants hold a prognostic value regarding treatment response, where e14a2 predicts a faster and deeper treatment response. However, no difference in overall survival has been observed and the issue remains controversial. Reverse transcription quantitative PCR (RT-qPCR) using the EAC qPCR assay has been the gold standard for determining the levels of BCR-ABL1 transcripts. The assay use common primers for amplification of the two variants resulting in a PCR product for the e14a2 variant that is 75 base pairs longer than the e13a2 variant. Under suboptimal PCR conditions, amplicons may be amplified with different efficiencies, which can result in an underestimation of especially the amount of longer qPCR products.

Aims: To study the accuracy of the EAC assay in quantifying the e13a2 and e14a2 transcripts.

Methods: Patient samples were screened for BCR-ABL1 e13a2 and e14a2 transcript variants using either PCR with agarose gel separation or a droplet digital PCR (ddPCR) assay measuring the amount of e13a2 and e14a2 transcripts. The BCR-ABL1 level was determined by qPCR using the QuantiStudio instrument (Life Technologies) and expressed in the International Scale (IS) using the EAC primers and assay conditions with GUSB and BCR as reference genes. Samples were re-measured by digital droplet PCR (ddPCR) on a Quantitect instrument (Bio-Rad) using modified EAC primers multiplexed with GUSB and BCR as reference genes and expressed as %IS.

Results: Transcript levels from 124 BCR-ABL1 positive patient samples were determined using the EAC qPCR assay (median: 0.08% IS; range: 0.001–159.0% IS) and ddPCR (median: 0.01% IS; range: 0.0002–124.4% IS). These included 59 samples with the e13a2 variant and 65 with the longer e14a2 variant. Comparing the expression levels obtained by the two techniques revealed ddPCR/qPCR ratio differences for e13a2 (median: 0.68, range: 0.35–3.2) and e14a2 (median: 3.43, range: 0–8.8), and a consistent 4.5 fold (>0.5 log) underestimation of the levels of the e14a2 compared to e13a2 when using qPCR (figure 1).

Summary/Conclusions: When we compared the BCR-ABL1 levels using qPCR and ddPCR, we observed a discrepancy between the e13a2 and e14a2 transcript variants. Since ddPCR is an endpoint measurement and not sensitive to variations in primer efficiencies, the most likely explanation for the discrepancy is a decreased qPCR efficiency of the longer e14a2 variant compared to e13a2 variant. Thus in qPCR analyses using the EAC protocol this may, at least in some analysis platforms, result in a consistently underestimation of the e14a2 level resulting in an appealingly better treatment response. A more than 0.5 log underestimation in a large subgroup of patients could have consequences in clinical decision-making e.g. by miss-grouping patients at different time points or when considering TKI discontinuation. Since many clinical laboratories use the EAC-protocol when considering TKI discontinuation, or laboratories uses the qPCR protocol when considering TKI discontinuation, it is important to know which of the protocols underestimates the BCR-ABL1 transcript levels more.

Results: Baseline characteristics of the CP-CML pts included: median time from diagnosis, 7 yrs (range, 0.5–27 yrs); median age, 60 yrs (18–94 yrs); median %Ph+, 100% (2.5–100%); ≤10% Ph+, 20 pts (7%); 60% of CP-CML pts received ≥3 prior TKIs. At initiation of study closure, 99 pts (37%) were ongoing; among these pts, minimum follow-up was 52 mos, and most (78%) had 15% residual BCR-ABL1 as their last ponatinib dose. In efficacy-evaluable CP-CML pts, cumulative response rates as of the data cutoff were: MCyR, 60%; CCyR, 54%; MMR, 40%; and MR4.5–complete (CCyR) at 3-6, and 12-mos with progression-free survival (PFS) and overall survival (OS) 4 yrs past landmark (log-rank P values). Data cutoff: 3 Oct ’16.

Table 1.

Figure 1.
P604

LONG-TERM FOLLOW-UP IN VERY ELDERLY PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH IMATINIB FRONTLINE

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Background: Very elderly (>75 yrs) people are a substantial proportion of chronic myeloid leukemia (CML) patients that sometimes receive Imatinib (IM) at reduced doses based on physicians’ judgment. However, data on long-term follow-up of these patients are still lacking.

Aims: To investigate the treatment response and outcome in a cohort of very elderly patients with newly diagnosed CML in chronic phase.

Methods: We revised in a retrospective database 263 CML, HIV, patients aged ≥75 years and diagnosed from 2/2002 to 1/2016 and treated with IM frontline; among these, 121 patients (46%) were older than 80 yrs. (68.4%), high in 78 (31.2%) and not evaluable in 13 patients. As regards concomitancies, 121 patients (46%) were older than 80 yrs.

Results: Median age at diagnosis was 78.5 yrs [interquartile range (IQR) 76.3–81.3]. Sokal Risk at diagnosis was low in 1 patient (0.4%), intermediate in 171 (68.4%), and high in 51 (20.1%) and not evaluable in 13 patients. As regards concomitancies, 63 patients had no or 1 concomitant disease, 47 patients 2 or 3 and 11% in 4 or more. Median interval from diagnosis to IM start was 0.8 month (IQR 0.3–1.6): the initial IM dose was 400mg/day in 180 (68.4%), 300mg/day in 67 (25.5%) and <300mg/day in 16 (6.1%) patients. According to WHO, 39 (15%) patients had a significant haematologic and 51 (19.4%) patients, respectively. As regards cumulative response, 13 patients (4.9%) discontinued IM due to early toxicity, 4 (1.5%) were resistant and 2 (0.8%) died from unrelated causes early after IM initiation; 250 patients (92.8%) achieved a complete haematological response (CHR). Among these, 208 (79% of all 263 patients) achieved a cytogenetic response (CyR), which was partial in 18 patients and complete (CCyR) in 190 (72.2%) after a median period of 6.1 months since IM initiation (IQR 3.4–9.1). Among the 190 patients in CCyR, 148 (56.2%) achieved a molecular response (MMoR) (ratio < 0.1) after a median period of 13.7 months (IQR 9.0–22.3). Eleven patients (4.2%) developed a blastic phase (myeloid in 8 and lymphoid in 3 cases). After a median follow-up of 45.0 months from IM start (IQR 22.3–72.0), 93 patients have died (9 from disease progression and 84 from unrelated causes), 144 are alive and 104 of them are still in treatment with IM, while 8 discontinued for prolonged deep molecular response and 22 switched to 2nd line TKI. Five-years event-free survival (EFS) and overall survival (OS) were 51.2% (CI95% 44.8-57.6) and 70.9% (CI95% 64.6-77.2), respectively. At univariate analysis Hb level at diagnosis (≥ 12 vs < 12g/dl, p=0.021) and the initial dose of IM (400 vs <300, p=0.048) were significant predictive factors for EFS. According to CyR achievement, while PLT and CyR diagnosis (< 15 x 10^9/l, p<0.006) and female gender (p=0.046) were significant predictive factors for MoIMoR achievement. Multivariate analysis for EFS and OS are described in Table1.

Table 1.

Summary/Conclusions: The long term follow-up of very elderly CML patients treated with IM suggests that any effort to treat these patients should be made, in order to achieve cytogenetic and molecular responses as in younger subjects.

P605

IMPACT OF ARTERIAL THROMBOTIC EVENTS ON THE LONG-TERM OUTCOME OF CHRONIC MYELOID LEUKEMIA (CML) PATIENTS TREATED WITH NILOTINIB: A PROSPECTIVE STUDY WITH NILOTINIB: AN ANALYSIS OF THE GIMEMA CML WORKING PARTY


Background: Nilotinib has shown better efficacy compared to imatinib, but it has been associated to a higher incidence of arterial thrombotic events (ATEs). The aim of this study is to determine the impact of ATEs on Treatment Response Rates and the long-term outcome of CML patients treated with nilotinib. Methods: We analyzed 345 patients ≥ 18 years of age with CP CML enrolled in clinical trials of the GIMEMA CML WP investigating nilotinib as first-line treatment. Patients were treated with: nilotinib 400mg BID (n=73); rotation of nilotinib 400mg BID / imatinib 400mg OD (3-month periods for each drug)(n=123); nilotinib 300mg BID (n=149). The median follow-up was 58 (22-82) months. The median age at CML diagnosis was 53 (18–86) years. We analyzed the rate, type, management, and outcome of ATEs; moreover, we compared the molecular response rates and the long-term outcome of CML patients treated with nilotinib. Results: Overall, 30/345 (8.7%) patients had ATEs during treatment with nilotinib. The median age at CML diagnosis of these patients was 64 (43-85) years, and the median age at ATEs was 67 (47-89) years. The median duration of nilotinib treatment at ATE was 25 (1-78) months. ATEs were: 14 coronary dis...
ease (including 7 acute myocardial infarction), 8 PAOD, 4 carotid stenosis (asymptomatic), 2 avascular necrosis of femoral head, 1 optic artery ischemia, 1 aneurysm of aorta/right iliac artery. Overall, 21 patients were hospitalized for the management of ATEs; 15 patients received medical treatment only, while the remaining required invasive interventions: 9 coronary angioplasty with stent positioning, 3 lower limbs amputations, 2 peripheral vascular bypasses, and 1 prosthesis of femoral head. No patient died for ATEs. Overall, 24 patients (80% of patients with ATEs, and 7% of the whole cohort) permanently discontinued nilotinib because of ATEs. The median follow-up after ATE was 15 (1–58) months. Of the 30 patients with ATEs, 26 (87%) achieved a MMR and 18 (60%) obtained a MR4, during nilotinib treatment. These rates were comparable to those observed in patients without ATEs (MMR: 260/315, 83%; MR4: 113/315, 64%). The 5-year progression-free survival and overall survival for patients with or without ATEs (PFS: 96% vs 92%, p=0.05; OS: 96% vs 93%, p=0.79).

Summary/Conclusions: After a median follow-up of 58 months, 8.7% of patients treated front-line with nilotinib had ATEs, being coronary disease and PAOD the most common. ATEs were more frequent in elderly patients (median age at ATEs: 67 years). Half of the patients required invasive procedures, including major surgeries in 6 patients. The other patients were successfully managed with medical treatment. Importantly, no patients died for ATEs, and ATEs did not affect the rates of MMR, MR4 and 5-year PFS and OS, which were all comparable to those observed in patients without ATEs. Taken together, these data suggests that ATEs, despite being sometimes associated with significant morbidity, did not significantly impact on response rates and on long-term outcome of CML patients treated with nilotinib front-line.

P606

ASSESSMENT OF CHRONIC RENAL INJURY IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN THE CHRONIC PHASE RECEIVING TYROSINE KINASE INHIBITORS

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Background: Long-term use of tyrosine kinase inhibitors (TKIs) may lead to chronic renal injury.

Aims: To evaluate the incidence of chronic kidney disease (CKD) in patients with chronic myeloid leukemia (CML) in the chronic phase (CP) receiving TKIs, and to identify the factors associated with the onset of CKD.

Methods: Data of CML-CP patients treated with TKIs as first-line or second- or third-line therapy for at least 3 months were analyzed. Glomerular filtration rate (GFR) was followed from the initiation of TKI-therapy. CKD was defined as persistent GFR < 60 ml/min/1.73 m2 or persistent more than 30% GFR reduction from baseline. CKD-free survival was used to evaluate the onset of CKD. Patients’ characteristics and TKI used were analyzed to identify the factors associated with the onset of CKD by Cox regression model in those receiving first-line and second- or third-line TKI-therapy, respectively.

Results: 587 patients were included in this study. 83% (65%) were male. Median age was 40 (17–84) years. 464 patients received imatinib (n=363), nilotinib (n=88) or dasatinib (n=13) as first-line TKI-therapy. With a median follow-up of 35 months (range, 3–185 months), 136 of 416 (33%) patients with normal GFR at baseline developed CKD. Probabilities of CKD-free survival at 4 years were 62%, 78% and 100% in the patients receiving imatinib, nilotinib and dasatinib, respectively (p=0.004). Multivariate analysis showed that imatinib use (HR=2.4, 95% CI 1.4-4.3, p=0.002), male gender (HR=2.0, 95% CI 1.4-2.9, p<0.001), increasing age (HR=1.2, 95% CI 1.1-1.4, p=0.003) and prior administration of hydroxyurea, interferon or chemotherapy (HR=1.7, 95% CI 1.1-2.8, p=0.010) were factors associated with incident of CKD. In 48 patients with abnormal GFR or prior CKD before first-line TKI-therapy, 8 of 42 (19%) developed ≥30% GFR reduction from baseline in the first 6 months, while none of 6 during nilotinib- or dasatinib-therapy. In 123 patients receiving nilotinib (n=59) or dasatinib (n=64) as second- or third-line TKI-therapy after imatinib-failure, 13 of 110 (12%) with normal GFR at baseline developed CKD with a median follow-up of 19 months (range, 3-149 months). Probabilities of CKD-free survival at 3 years were 74% and 90% in those receiving nilotinib and dasatinib, respectively (p=0.059). Multivariate analysis showed that nilotinib use (HR=3.6, 95% CI 1.6-8.3, p=0.047) and a history of diabetes mellitus, hypertension or other renal diseases (HR=3.8, 95% CI 1.3–11.6, p=0.019) were factors associated with incident of CKD. 3 of 13 (23%) patients with abnormal GFR or prior CKD before second- or third-line TKI-therapy developed ≥30% GFR reduction from baseline during nilotinib (n=1) or dasatinib (n=2) therapy.

Summary/Conclusions: Our study showed that nilotinib and dasatinib were associated with less chronic renal injury compared with imatinib as first-line TKI-therapy, while dasatinib was related to less loss of renal function compared with nilotinib as second- or third-line TKI-therapy after imatinib-failure in CML-CP patients.

P607

COMPARATIVE MONITORING OF MINIMAL RESIDUAL DISEASE (MRD) BY qPCR AND dPCR IN CHRONIC MYELOID LEUKEMIA PATIENTS ACHIEVING MAJOR OR DEEP MOLECULAR RESPONSE WITH TIROSIN-KINASE INHIBITORS

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Background: Quantification of BCR-ABL1 transcript by qPCR is mandatory to monitor the response to TKIs therapy in CML patients. The achievement of Major or Deep Molecular Response (MMR or DMR) with TKIs is crucial for long-term survival and for treatment free remission (TFR). Currently, up to 30-40% of CML patients treated with TKIs can achieve DMR, but 50-60% of responders who discontinue the treatment lose their DMR and re-challenge continuous TKIs therapy. qPCR has some intrinsic limitations and it does not appear to be an optimal assay to select the best candidates to TKIs discontinuation. Digital PCR (dPCR) can give an absolute quantification of target nucleic acids by partitioning the PCR reaction mix over a large number of wells, each containing a single copy or no copies of the target region.

Aims: The aim was to comparatively monitor the BCR-ABL1 transcript levels by dPCR and qPCR in 57 CML patients treated with TKIs and achieving MMR or DMR in at least 3 time points.

Methods: Using qPCR and dPCR (Q30 Digital PCR System by Life Technologies), we comparatively analyzed 228 peripheral blood samples from 57 CML patients with MMR (n=14) or DMR (n=43). qPCR analysis were performed according to the last International Guidelines while absolute quantification of BCR-ABL1 transcript were obtained by dPCR and results were expressed as number of BCR-ABL1 copies/ul of reaction. Patients were divided into 3 groups corresponding to the MR classes at the first time point: MR3.0, MR4.0 and MR4.5-5.0 groups. dPCR Positive Predictive Value (PPV) was also preliminary evaluated in 14 patients undergoing TKI discontinuation.

Results: Analyzing comparatively the time course of MR in the patients of the three groups (MR3.0, MR4.0 and MR4.5-5.0) it was observed a similar trend, but the dPCR allowed to appreciate that, at the time of starting the monitoring the patients showed different levels of BCR-ABL1 copies/ml. Furthermore, those patients with MR4.5-5.0 undetectable by qPCR resulted with detectable BCR-ABL1 transcript levels when assessed by dPCR. Secondly, while MRD quantitations measured by qPCR appear to be more homogeneous, nearly due to a normalization effect of qPCR, the quantitations of MRD measured by dPCR appear to be more heterogeneous because of the high sensitivity and accuracy of dPCR. Therefore, dPCR values, reflecting the great heterogeneity of MRD level in patients belonging to the same MR group, suggest a higher accuracy in patients stratification (Figure 1a). dPCR value of 0.468 copies/ul, previously reported as value discriminating between major responders and deep responders, was used as a threshold for dPCR data analysis. Patients with absolute value of BCR-ABL1 lower than 0.468copies/ul at the first time point presented more stable disease levels than the patients with absolute value of BCR-ABL1 higher than 0.468copies/ul (Figure 1b). In 14 CML patients who
OUTCOME OF BLAST PHASE CHRONIC MYELOID LEUKEMIA (CML-BP) IN THE TYROSINE KINASE INHIBITOR ERA

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Background: Primary goal of management in chronic myeloid leukemia (CML) is to prevent disease progression to blast phase CML (BP-CML). Current notion for management of BP-CML usually involves initiation of intensive chemotherapy regimen with addition of tyrosine kinase inhibitor (TKI). Despite treatment with intensive induction chemotherapy, outcome remains dismal.

Aims: We aimed to describe our experience with management of BP-CML and its outcome.

Methods: We included 58 patients from Moffitt Cancer Center from 2001 till 2016 with diagnosis of BP-CML and performed a retrospective chart review. Data elements including age, gender, peripheral blood and bone marrow parameters, phase of CML, treatment, cytogenetics and vital status were collected. Survival analysis using Kaplan-Meier method with log-rank test to determine significance by calculating two-sided p values was performed.

Results: The overall survival (OS) of our cohort was 31.87 months (mo). For patients with progression to BP-CML from previously known diagnosis of CML, median time to progression was 19.1 mo (range: 3.0-221.2 mo). The median OS from the diagnosis of BP-CML in this cohort was 10.8 mo, compared to lymphoid blast phase OS of 11.03 mo (p value=0.62). Myeloid blast phase CML had worse OS compared to lymphoid blast phase cohort but was not statistically significant (9.17 vs 17.5 mo, p=0.32). We further compared the treatment strategies of BP-CML including single agent TKI (n=21) and conventional chemotherapy regimens in combination with a TKI (n=36). The median OS of the cohort with single agent TKI was not statistically different from the combination with chemotherapy arm (12.83 mo vs 10.87 mo, p=0.73) as shown in Figure 1A. Additionally, combination of chemotherapy with TKI compared to single agent TKI did not have significant survival impact in either myeloid (9.17 vs 9.13 months, p=0.32) or lymphoid (14.47 vs 18.27 mo, p=0.24) BP-CML. Total of 26 patients (44.8%) proceeded to allogeneic bone marrow transplant, 26% (n=6) of which only received TKI prior to transplant compared to 76.9% (n=20) who received chemotherapy in combination with TKI. Use of single agent TKI rather than TKI in combination with chemotherapy prior to allogeneic transplant had a trend toward improved OS (128.5 vs 24 mo, p=0.23) (Fig 1B). Choice of TKI in combination with chemotherapy in treatment of BP-CML also did not identify any TKI combination resulting in superior survival (Figure 1D). Overall survival of the cohort stratified by presence of standard Philadelphia chromosome in comparison to additional cytogenetic aberrations did not detect difference in overall survival (10.87 vs 12.1 mo, p=0.51). Further evaluation of cytogenetic aberrations revealed monosomy 7 to be present in greater frequency in lymphoid blast phase compared to myeloid blast phase (35.71% vs 6.25%, p=0.02).

Conclusion: We data suggest no survival difference when BP-CML is treated with a single agent TKI compared to a combination therapy, regardless of histology type. Therefore, single agent TKIs should be considered as an effective frontline therapy option for BP-CML, which may prevent the potential toxicity associated with chemotherapy. These findings need further validation in a larger prospective cohort.

Efficacy of Switching to Dasatinib in Chronic Myeloid Patients with Late Warning Responses to Imatinib. Study of the Association of Response to Dasatinib to Immunologic Status

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Background: European LeukemiaNet (ELN) recommendations (2013) advised closely monitoring for patients with late warning response (patients with complete cytogenetic response without major molecular response after 12 months of treatment). Our trial, DASAPOST, has been the first one evaluating efficacy and safety of dasatinib in patients with late warning responses, and preliminary results have been reported (García-Gutierrez et al, ASH 2016; P5450). Besides, many studies suggest that dasatinib may augment responses due to its immunomodulating effect. Although NK and CD8 cells seem to be involved, the specific mechanism remains to be clarified.

Aims: To evaluate the efficacy and safety of switching change to dasatinib in patients treated with imatinib first line during at least 18 months and having a late warning response, and to study the association between response to dasatinib and immune robustness, both baseline and during the therapy, and dasatinib-induced lymphocyte mobilization.

Methods: Phase II, open, multicenter DASAPOST study (NCT01802450). Patients previously treated with imatinib after at least 18 months, with CCyR but without MMR, were included. All BCR-ABL1/ABL (IS) measurements were centrally standardized in a EUTOS laboratory. Patients not molecularly analyzed at a given time point were considered as non responders. Lymphocyte counts, subpopulations and migration studies were done at baseline (1st day of dasatinib), and every 3 months, and 6 months after dasatinib discontinuation, a preliminary analysis showed 80% of patient with BCR-ABL1=0.64 copies/ul at discontinuation, maintained stable TFR (PPV of 80%).

Summary/Conclusions: Results from April 2013 to May 2015, 18 patients were enrolled in 12 centers. Median age was 59 years (39-77). The ratio of men to women was 13/5, and the Sokal risk groups were 48%, 30% and 22% for low, intermediate and high risk, respectively. Median time from diagnosis to switch to dasatinib was 2.6 years (1.6-23) and median time while on imatinib to achieve CCyR 1.4 years (0.2-12). Median exposure to imatinib was 2.4 years (1.6-14). Eight patients (44%) attained MMR at 3 months, and 12 (66.7%) obtained MMR at 6 and 12 months. Of interest 9/18 patients (50%) achieved MR4 by 12 months. There were 3 study discontinuations because of toxicity (16%). Table 1 shows the median number of the most relevant lymphocyte populations in the pre-dose sample at baseline. Table 2 shows that the absolute number of CD8 cells was significantly superior at baseline in those patients having a MM at 3 months, with a trend in the same direction of absolute lymphocyte count and percentage. There were no significant associations with response when considering CD4 T cells, NK cells, or the degree of mobilization after dasatinib dose either in total lymphocyte numbers or in subpopulations. Besides, lymphocyte number or proportions at 3 or 6 months were not associated with MMR at 6 or 12 months (data not shown).

Table 1.

<table>
<thead>
<tr>
<th>Lymphocytes Baseline</th>
<th>CD8 Baseline</th>
<th>CD4 Baseline</th>
<th>NK Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (x 10^3/L)</td>
<td>(x 10^3/L)</td>
<td>(x 10^3/L)</td>
<td></td>
</tr>
<tr>
<td>Percentage</td>
<td>(10.4±2.7)</td>
<td>(10.4±2.7)</td>
<td>(10.4±2.7)</td>
</tr>
</tbody>
</table>

Table 2.

<table>
<thead>
<tr>
<th>Lymphocytes Baseline (x 10^3/L)</th>
<th>MMR 3m</th>
<th>No MMR 3m</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8 Baseline (%)</td>
<td>32.4</td>
<td>25.1</td>
<td>0.03</td>
</tr>
<tr>
<td>MMR 3 months (x 10^3/L)</td>
<td>0.67</td>
<td>0.49</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Results: From April 2013 to May 2015, 18 patients were enrolled in 12 centers. Median age was 59 years (39-77). The ratio of men to women was 13/5, and the Sokal risk groups were 48%, 30% and 22% for low, intermediate and high risk, respectively. Median time from diagnosis to switch to dasatinib was 2.6 years (1.6-23) and median time while on imatinib to achieve CCyR 1.4 years (0.2-12). Median exposure to imatinib was 2.4 years (1.6-14). Eight patients (44%) attained MMR at 3 months, and 12 (66.7%) obtained MMR at 6 and 12 months. Of interest 9/18 patients (50%) achieved MR4 by 12 months. There were 3 study discontinuations because of toxicity (16%). Table 1 shows the median number of the most relevant lymphocyte populations in the pre-dose sample at baseline. Table 2 shows that the absolute number of CD8 cells was significantly superior at baseline in those patients having a MMR at 3 months, with a trend in the same direction of absolute lymphocyte count and percentage. There were no significant associations with response when considering CD4 T cells, NK cells, or the degree of mobilization after dasatinib dose either in total lymphocyte numbers or in subpopulations. Besides, lymphocyte number or proportions at 3 or 6 months were not associated with MMR at 6 or 12 months (data not shown).
Summary/Conclusions: Our study shows that in patients treated with imatinib and with late warning responses, switching to Dasatinib induced MMR in 2 out every 3 patients, and MR4 in half of the patients, with a good safety profile. Contrarily to other group reports, we have not found any significant association between response and lymphocyte mobilization in any point studied. Interestingly, the absolute number of CD8 at baseline was significantly associated with the early attainment of MMR at 3 months, a finding which underscore the prognostic importance of baseline immune status, the relevance of CD8 cells in the antileukemic effect, and which suggest that this quite simple variable must be included in future studies with dasatinib in second line.

Methods: The BCR-ABL measurements acquired using EAC and in-house modified EAC protocol have been compared with results from SA Pathology in Adelaide. The Adelaide protocol (Branford and Hughes 2006) consists of separate, optimized reactions for e13a2 and 14a2 transcripts, therefore it should be considered free of any PCR efficiency-related artifacts. The data originated from four independent sample batches exchanged between Poznan and Adelaide since 2009.

Results: The analysis of retrospective EAC protocol data showed that when e13a2 and e14a2 samples entered the exponential phase at the same time, the latter would cross the threshold approximately 2.2 cycles after the first one. Re-analysis of data from sample exchanges from 2009 revealed that after establishing a conversion factor (CF), all of the e14a2 measurements in Poznan were underestimated according to Adelaide. At the same time, almost all of e13a2 samples were overestimated (fig. 1). Still, the bias between methods was acceptable and a valid conversion factor (CF) was calculated. The method modification introduced 2011 eliminated this difference and increased concordance between laboratories. The last sample batch revealed significant difference between non-modified and modified EAC protocols in e13a2 measurements: 4.56 (+/- 0.96). Reanalysis of sample batch from 2009 (presented on fig.1) using 4.57 (2x2.28) factor (e13a2 results divided by 2.28, e14a2 results multiplied by 2.28) resulted in almost perfect data alignment. The results of modified EAC protocol, after CF recalculation, showed very good concordance with Adelaide (100% results of e14a2 and 88% of e13a2 within 2-fold of reference laboratory).

Figure 1.

Summary/Conclusions: In the EAC protocol, the e14a2 transcript amplifies less efficiently than e13a2. Since commonly used plasmids, including ERM-AD623, are based on e14a2, the standard curve is being shifted towards the latter cycles. It leads to overestimation of e13a2 by mean factor of 4.5 (over 0.5 log), which could be clinically significant. The reports of worse outcome of e13a2 patients are probably caused by this artifact, which can be easily eliminated by implementing an additional forward primer to EAC protocol. This overestimation cannot be detected in case of lab to lab validation when two centers are using EAC protocol. In case of method validation in Adelaide, those differences were not as obvious as well. The shift of 4.5 (fig. 1) means that results are 2.25 times different from the perfect concordance line and could easily fit into accepted 2-fold and 3-fold compartments. The CF calculated by Adelaide would depend on the percentage of each transcripts among the exchanged samples. The observed artifact should be also taken into consideration in clinical trials that rely on surrogate endpoints such as molecular response level at certain time points. Uneven transcript variant distribution between compared groups may lead to improper conclusions.
Enzymes and sickle cell disease

**P612**

**ESTABLISHMENT OF IN VIVO AND IN VITRO MODEL OF X-LINKED SIDEROBlastic ANEMIA**

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**Background:** Congenital sideroblastic anemia (CSA) is a inherited congenital anemia characterized by the presence of bone marrow ring sideroblasts, reflecting excess mitochondrial iron deposition. The most common form of CSA is X-linked sideroblastic anemia (XLSA), which is attributed to mutations in the X-linked gene erythroid-specific 5-aminolevulinate synthase (ALAS2). ALAS2 resides on chromosome X and encodes the enzyme that catalyzes the first and rate-limiting steps in the heme biosynthesis pathway in erythroid cells. This pathway converts glycine and acetyl-coenzyme A to 5-aminolevulinic acid (ALA), which requires pyridoxal 5'-phosphate (PLP) as a cofactor. Although ALAS2 lacks PLP has been used for treating XLSA, a marked proportion of patients with XLSA remain refractory to treatment (Ohta et al. Ann Hematol 2013). Thus, there is a need to establish a model of XLSA to reveal the detailed molecular mechanism contributing to RS formation as well as to explore novel therapeutic strategies for XLSA.

**Aims:** We explored to establish a novel model of XLSA by CRISPR/Cas9-based genome editing.

**Methods:** We targeted the GATA-1-binding region of intron 1 of the human ALAS2 gene based on both in vivo mice and human induced pluripotent stem cell-derived erythroid progenitor (HiDEP) cells (Kurita et al. PLoS One 2013). The mutation diminished the binding of transcription factor GATA-1, which would lead to decreased transcription of the ALAS2 gene, thereby causing XLSA (Kaneko et al. Haematologica 2014). Western blotting and quantitative chromatin immunoprecipitation (ChIP) analysis were performed using antibodies against GATA-1 (D5S246, Cell Signaling Technologies) and TAL1 (C-21, Santa Cruz). For transcription profiling, Human Oligo chip 25K (Toray) was used. Microarray analysis revealed >2-fold up- and down-regulation of 619 and 274 genes caused by the 19-bp deletion, respectively. The significant decreased intracellular heme concentration. Despite significant downregulation of HBA1, HBE1, HBM1, and HBB as well as globin genes (HBA1, HBE1, HBM1, and HBB) as well as genes involved in iron/heme metabolism (ALAS2, transferrin receptor, FTRC, coproporphyrinogen oxidase, CPOX, and mitoferrin 1; MFRN1), GO analysis revealed significant enrichment of cellular iron homeostasis (p=0.018), regulation of translation (p=0.0021), and innate immune response (p=0.0018), implying that heme was involved in various biological processes in erythroid cells. Interestingly, ALA treatment significantly improved compromised heme production as well as downregulation of globin genes observed in the XLSA clone, suggesting that ALA may represent a novel therapeutic option for PLP-refractory XLSA.

**Summary/Conclusions:** The XLSA model established from HiDEP cells can be used as an important tool for clarifying the molecular etiology of XLSA and to explore novel therapeutic strategies.

**P613**

**BENDAMUSTINE AND RITUXIMAB COMBINATION THERAPY FOR COLD AGGLUTININ DISEASE:**

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**Background:** Primary cold agglutinin disease (CAD) is an autoimmune hemolytic anemia in which a well-defined clonal lymphoproliferative bone marrow disorder (LPD) causes production of monoclonal cold agglutinins. Major clinical manifestations are anemia and, less frequently, cold-induced circulatory symptoms. Pharmacological therapy, although not indicated in patients with very mild disease, seems required in a majority of cases. Corticosteroids are ineffective. Rituximab monotherapy has resulted in approximately 50% response rate and 1-year median response duration. Fluadrabine and rituximab combination therapy showed 70% response rate (20% complete responses) and very long response duration, but considerable toxicity.

**Aims:** We wanted to investigate whether bendamustine and rituximab combination therapy can result in favorable response rates and duration with an acceptable toxicity profile.

**Methods:** We conducted a prospective, uncontrolled multicenter trial with 16 participating hospitals from Norway, Finland and Denmark. Essential inclusion criteria were verified CAD with symptomatic anemia and/or severe cold-induced circulatory symptoms. Eligible patients received 4 cycles of rituximab 375mg/m² day 1 and bendamustine 90mg/m²/day 1-2 with 28 days interval. Outcomes were evaluated into complete response (CR), partial response (PR), non-response (NR). The definition of CR included normalization of hemoglobin (Hb) levels with no hemolysis, complete histologic resolution of the bone marrow LPD and disappearance of monoclonal serum protein. The criteria for PR included increase in Hb levels by at least 2.0 g/dL or to the normal range, transfusion independence, at least 50% reduction of IgM and improvement of any circulatory symptoms.

**Results:** Forty-four patients (19 men and 25 women) were included, with a median age of 74 years (range, 48-86) and median disease duration 4 years (range, 0–16). Seventeen patients had received previous therapy. At baseline, median Hb level was 9.5g/dL (range, 4.5–14.5), bilirubin 45micromol/L, lactate dehydrogenase (LDH) 468 U/L, haptoglobin undetectable, IgM 4.1g/L (1.0-27.2), CA104 2084 (64-65536). Monoclonal IgM kappa was detected in 38 patients, IgG kappa in 1 and IgA kappa in 1. We observed CR in 16 patients (36%), PR in 15 (34%), while the remaining 13 (30%) were non-responders. Hb levels were increased in median of 4 g/dL in the responders; 4 g/dL in patients achieving CR and 3.9g/dL in those achieving PR. Median post-therapy Hb levels were 14.2g/dL (CR), 12.5g/dL (PR) and 10.5g/dL (NR). Acrocyanosis and Raynaud symptoms resolved completely in 16 patients and improved in 11 (47% and 32%, respectively, of those with such symptoms at baseline). Histologic regression of the LPD was complete in 17 patients (39%), partial in 5 (11%) and not evaluable in 18 (41%). Median time to response was 2 months (0.5-12). Only 3 responders experienced relapse; 2 after PR and 1 after CR. Median observed response duration was 32 months (range, 1-62) during median 32 months follow-up. The overall response, complete or partial, was a much longer median of 39 months. Neutropenia grade >3 occurred in 14 patients (32%), of which 8 (18%) had grade 4. Three patients (7%) experienced 1-3 episodes of febrile neutropenia, which was readily manageable. Non-hematologic toxicity occurred in 17 patients (39%), mostly consisting of mild nausea or rash. Three non-neutropenic serious adverse events (SAE) were recorded; 1 was considered probably therapeutically related.

**Summary/Conclusions:** Bendamustine and rituximab combination therapy resulted in high response rates, a high rate of CR, long response duration and few relapses during the observation period, with a favorable safety profile. It might be considered in the first line for reasonably fit patients with CAD requiring therapy.

**P614**

**EX VIVO TREATMENT OF RED BLOOD CELLS FROM 15 PYRUVATE KINASE (PK)-DEFICIENT PATIENTS WITH AG-348, AN ALLOSTERIC ACTIVATOR OF PK-R, INCREASES ENZYMATIC ACTIVITY, PROTEIN STABILITY AND ATP LEVELS**

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**Background:** Pyruvate kinase (PK) deficiency is a rare hereditary disorder affecting red blood cell (RBC) glycolysis. It is caused by mutations in the PKLR gene. PK-deficient RBCs are characterized by changes in metabolism associated with defective glycolysis, including a build-up of the upstream metabolite 2,3-diphosphoglycerate, and deficiency in the PK product ATP. It is hypothesized that insufficient energy production affects red cell homeostasis, promoting haematologica | 2017; 102(s2) | 241
premature removal of PK-deficient RBCs from the circulation. Affected patients display chronic hemolytic anemia of variable severity. Treatment of PK-deficient patients is generally supportive, focusing on the anemia and iron overload state, and there are no approved drugs that directly target mutated PK. AG-348 is an allosteric activator of the RBC isoform of PK (PK-R) and in clinical development for the treatment of PK deficiency.

**Aims:** To evaluate the effect of AG-348 treatment on PK-R enzymatic function, RBC metabolism and deformability.

**Methods:** Observational case-control study, approved by the Institutional Review Board. All patients gave informed consent. Enrolled patients (N=15) were adults, transfusion-independent and compound heterozygous or homozygous for PKLR genotypes. Baseline RBC metabolic profiling was performed by LC-MS/MS. Purified RBCs from patients and healthy control subjects were incubated with AG-348 (up to 10 μM) for 24 hours at 37°C. After 6 and 24 hours PK-R activity, ATP levels and RBC deformability (by Lorcca) were measured. For determination of PK-R thermal stability, RBC lysates were incubated for 2 hours with 2 μM AG-348 at 53°C) prior to testing. Baseline protein levels of PK-R were assessed using antibodies against PK-R.

**Results:** Baseline patient characteristics show strongly reduced PK-R activity in all patient cells, in particular taking into account the degree of reticulocytosis (Table 1). Distinct metabolic changes were consistent with a block of glycolysis at PK-R step. Treatment of PK-deficient RBCs with AG-348 resulted in increased enzymatic activity in all patient cells after 24 hours (mean increase 1.8-fold, range 1.2-3.4). Similar increases were observed in control cells (mean fold increase 2.3, range 1.2-7.1). ATP levels in PK-deficient cells increased upon AG-348 treatment (mean fold increase 1.5-fold, range 0.2-2.2) similar to controls (mean increase 1.6 fold, range 1.4-1.8). Generally, PK-R thermal stability was strongly reduced in PK-deficient patient cells, illustrated by a mean loss of activity of 72% (19% for control cells) after incubation at 53°C for 60 minutes. Ex vivo treatment with AG-348 prior to incubation resulted in residual activity 4 to >10-fold higher than residual activity of vehicle-treated samples. Baseline protein level analyses suggests that a certain level of PK-R protein is required for cells to respond to AG-348 treatment ex-vivo, as treatment effects were minimal in patient cells with very low or undetectable levels of PK-R. In approximately half of the patients, ex vivo treatment with AG-348 was associated with an increase in RBC deformability, although there doesn’t appear to be a clear correlation with enzymatic or metabolic response.

**Summary/Conclusions:** These data support the hypothesis that drug intervention with AG-348 effectively upregulates PK-R enzymatic activity and increases stability in PK-deficient RBCs over a broad range of PKLR genotypes. The concomitant increase in ATP levels with 2 μM AG-348 suggests that glycolytic pathway activity may be restored. AG-348 treatment may represent an attractive way to correct PK-R dysfunction, and to achieve PK-R-related RBC deformability.

**P615**

**IDENTIFICATION OF NEW PATHOGENIC MUTATIONS IN PATIENTS WITH RED BLOOD CELL MEMBRANE DISORDERS USING NEXT-GENERATION SEQUENCING**

M.D.M. Mañu Pereira1,*, E. LLaudet Planas1, V. Rizzuto1, J. L. Vives Corrons1

**Background:** Red blood cell (RBC) membrane proteins deficiency or structural alterations lead to RBC membrane disorders such as hereditary spherocytosis, hereditary elliptocytosis or hereditary xerocytosis among others. Genetic analysis of RBC membrane proteins was performed by LC-MS/MS. Purified RBCs from patients and healthy control subjects were incubated with AG-348 (up to 10 μM) for 24 hours at 37°C. After 6 and 24 hours PK-R activity, ATP levels and RBC deformability (by Lorcca) were measured. For determination of PK-R thermal stability, RBC lysates were incubated for 2 hours with 2 μM AG-348 at 53°C) prior to testing. Baseline protein levels of PK-R were assessed using antibodies against PK-R.

**Results:** Baseline patient characteristics show strongly reduced PK-R activity in all patient cells, in particular taking into account the degree of reticulocytosis (Table 1). Distinct metabolic changes were consistent with a block of glycolysis at PK-R step. Treatment of PK-deficient RBCs with AG-348 resulted in increased enzymatic activity in all patient cells after 24 hours (mean increase 1.8-fold, range 1.2-3.4). Similar increases were observed in control cells (mean fold increase 2.3, range 1.2-7.1). ATP levels in PK-deficient cells increased upon AG-348 treatment (mean fold increase 1.5-fold, range 0.2-2.2) similar to controls (mean increase 1.6 fold, range 1.4-1.8). Generally, PK-R thermal stability was strongly reduced in PK-deficient patient cells, illustrated by a mean loss of activity of 72% (19% for control cells) after incubation at 53°C for 60 minutes. Ex vivo treatment with AG-348 prior to incubation resulted in residual activity 4 to >10-fold higher than residual activity of vehicle-treated samples. Baseline protein level analyses suggests that a certain level of PK-R protein is required for cells to respond to AG-348 treatment ex-vivo, as treatment effects were minimal in patient cells with very low or undetectable levels of PK-R. In approximately half of the patients, ex vivo treatment with AG-348 was associated with an increase in RBC deformability, although there doesn’t appear to be a clear correlation with enzymatic or metabolic response.

**Summary/Conclusions:** These data support the hypothesis that drug intervention with AG-348 effectively upregulates PK-R enzymatic activity and increases stability in PK-deficient RBCs over a broad range of PKLR genotypes. The concomitant increase in ATP levels with 2 μM AG-348 suggests that glycolytic pathway activity may be restored. AG-348 treatment may represent an attractive way to correct PK-R dysfunction, and to achieve PK-R-related RBC deformability.

**P616**

**CLINICAL FOLLOW-UP OF 378 PATIENTS WITH AUTOIMMUNE HEMOLYTIC ANEMIA: PROGNOSTIC IMPACT OF HEMOGLOBIN LEVELS, AUTOANTIBODY CLASS, AND RETICULOCYTOPENIA AT ONSET ON THE RELAPSE RISK AND OUTCOME**

B. Fattizzo1,*, A. Zaninoni2, J. Giannotta2, M. Lunghi3, A. Ferrari4, A.P. Lepori5, N. Mutucchio6, L. Scarescu7, G. Rossi8, G. Chiaruzzi9, D. (103/116) of the included patients could be determined and only in 11% (13/116) the mutation was not identified or the variant correlation with disease was not clear. It is worthy to highlight that 10 of the 13 undiagnosed patients had been oriented as unclear membranopathy.

**Conclusions:** According to the results, there is a high genetic heterogeneity in patients with RBC membrane disorders, as almost each family carries a unique mutation that is not observed in any other no related family. The present study reveals the usefulness of NGS panel, which allows the molecular diagnosis of almost the 90% of the patients and it would avoid misdiagnosis, and could lead to splenectomy in cases of severe inherited xerocytosis. Moreover, the 11% of undiagnosed patients will be analyzed through a second NGS gene panel including potential new genes leading to chronic haemolyosis and/or sequenced by whole exome sequencing with the aim to identify new disease causing genes.

**Table 1.** Baseline characteristics and genotypes of PK-deficient patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Phenotype</th>
<th>PKLR genotype</th>
<th>Hb (g/dl)</th>
<th>RBC count (x10^12/L)</th>
<th>Platelet (x10^12/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P01</td>
<td>Spherocytosis</td>
<td>c.567_568insC</td>
<td>6.5</td>
<td>3.2</td>
<td>169</td>
</tr>
<tr>
<td>P02</td>
<td>Spherocytosis</td>
<td>c.1274_1275delAC</td>
<td>7.0</td>
<td>4.8</td>
<td>230</td>
</tr>
<tr>
<td>P03</td>
<td>Spherocytosis</td>
<td>c.1274_1275delAC</td>
<td>7.5</td>
<td>5.2</td>
<td>250</td>
</tr>
</tbody>
</table>

**References:**

Results: Table 1 shows clinical and laboratory characteristics of AIHA cases at onset and distribution of thermal types. Hb values were significantly lower in IgG+C wAIHA and atypical cases (p<0.001), LDH higher in IgG+C wAIHA, mixed and atypical forms (p=0.01), and Hb and LDH values were negatively correlated (r=−0.25, p=0.001). Absolute reticulocytes were reduced in CAD, mixed and IgG+C wAIHA (p<0.001) together with inadequate reticulocytosis (p=0.01). Moreover, the reticulocyte index was lower in cases with Hb<6 g/dL (65 vs 98, p<0.001), along with more frequent inadequate reticulocytosis (87 vs 70%, p=0.01). First line therapy was administered in almost all cases but 25 CAD. A second therapy line was mostly required in IgG+C wAIHA, mixed, and to a lesser extent in CAD (p=0.005). The ultra-refractory cases requiring 4 or more lines of therapy were mainly mixed, atypical and CAD. Considering anemia severity, patients with Hb<8 g/dL more frequently required treatment after first-line (51 vs 33%, p=0.004; p=0.03), or even 3 or more therapy lines (52/71, 73% vs 19/71, 26%, p<0.001). The following hazard ratios (HR) emerged from multivariate Cox regression analysis: HR 3.2 (95% CI 1.4–7.2, 2.9 (1.4–6.2) 3.4 (1.6–7.5), for Hb <6, 6–8, and 8–10 g/dL compared to patients with Hb >10, respectively; as regards complications, infections were observed in 14% of cases, mostly mixed AIHA (p=0.02); thrombosis occurred in 10% and acute renal failure in 3% of patients, with no relationship with AIHA type/Hb values. Evans’ syndrome was more frequent in mixed or atypical cases (p=0.04) and in severe forms (74% with Hb<8 g/dL vs 26%, p=0.005), and was associated with higher relapse risk (HR 2.3, 95% CI 1.4–3.9). Seventy patients died during the follow-up, and 12 because of AIHA-related acute complications. Higher mortality was observed for infections (HR 5.8, 95% CI), acute renal failure (HR 7.6, 95% CI) and Evans’ syndrome (HR 8.3, 95% CI).

Summary/Conclusions: In conclusion, we found that severity of anemia at onset was the major determinant of relapse risk. The lowest Hb levels were observed in patients with IgG+C wAIHA and atypical cases along with higher LDH levels and inadequate reticulocytosis, advising strict clinical observation in these patients.

P617

HEMINE BINDS ANNEXIN-A5 DURING HEMOLYSIS AND PREVENTS ITS INTERACTION WITH CELL MEMBRANE PHOSPHATIDYLSERINE DURING SICKLE CELL DISEASE

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Background: Intravascular hemolysis, such as in sickle cell disease (SCD), is characterized by the damage of red blood cells resulting in hemoglobinuria, cell-free heme and extracellular vesicles in plasma, along with inflammation and tissue injury. Stressed leukocytes, platelets, endothelial and red blood cells shed microparticles (MP) that bear externalized phosphatidylserine (PS) at their surface and promote tissue injury. Conversely, intracellular annexin-A5 acts as an inhibitor of externalization of PS at the surface of cells and MP. Annexin-A5 is thound to orchestrate vesicle trafficking, promote cell membrane repair, protect against PS-mediated effects and enforce anti-inflammatory and anti-thrombotic control.

Aims: We investigated a possible functional relationship between intravascular hemolysis and annexins. We hypothesized that annexin-A5 in particular, is blocked by intracellular heme as it is released in plasma during intravascular hemolysis.

Methods: In order to test the heme-annexin-A5 relationship, we measured PS+, PS–, CD235a+ and annexin-A5+ circulating MP in adult SCD patient and matched control plasmas. We explored annexin-A5 expression in plasma and blood cells by Western blots and ELISA, and also quantified the PS-binding functionality of plasma annexin-A5 using a self-designed immunocapture assay and purified PS+ MP. Moreover, we investigated molecular interactions between purified heme and recombinant human annexin-A5 by surface plasmon resonance (BioChrom and Protein), absorbance shift assay and protein autofluorescence (Zeiss Axiovert 135TV). Finally, we put forward a model of heme-annexin-A5 docking by 3D molecular rendering.

Results: Immunocapture of plasma annexin-A5 revealed an association with heme (Abs398 nm signature) during SCD, especially during acute hemolytic events. In SCD plasma, we found increased total annexin-A5, but virtually undetectable levels of functional annexin-A5, contrary to controls. This implied a greatly reduced ratio of functional annexin-A5/circulating PS+ MP. Moreover, purified heme bond readily to annexin-A5 with relatively high affinity in vitro, as demonstrated using absorbance shift, autofluorescence quenching and plasmon resonance assays. With human serum albumin and hemopexin in competition, annexin-A5 bound one and two times as well as PS, respectively. We put forward a model of heme-annexin-A5 docking during SCD, especially during acute hemolytic events.

P618

USE OF PEGYLATED-CARBOXYHEMOGLOBIN BOVINE FOR THE TREATMENT OF SICKLE CELL DISEASE ASSOCIATED LEG ULCERS: RESULTS FROM A PHASE 2 SAFETY STUDY

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Background: Leg ulcers are a common complication of sickle cell disease (SCD). The pathophysiology of SCD leg ulcer is complex and may include obstruction of blood vessels by sickled red cell, chronic anemia, depleted nitric oxide bioavailability (resulting in impaired endothelial function), infection, thrombosis and excessive vasoconstriction. These events lead to progressive peripheral vascular obstruction, chronic tissue necrosis and ulceration, which eventually can become persistent ulcers, with no tendency to heal after months of appropriate treatment. PEGylated-Carbonyhemoglobin bovine (PEG-COHb; SANGUINATE) is an oxygen carrying agent with anti-inflammatory activity. A study of safety and effectiveness was undertaken in SCD patients with chronic leg ulcers to determine the safety of this investigational drug administered in as a once weekly infusion for either 4 or 6 weeks.

Aims: To assess the safety and efficacy of repeated doses of PEG-COHb on SCD leg ulcers.

Methods: The study was an escalating, repeated-dose, open-label, Phase 2 study to test PEG-COHb at 320mg/kg (8 mL) in subjects suffering from leg ulceration associated with SCD. It was conducted in Panama and the Dominican Republic. All enrolled subjects underwent a 3-week Run-In Period, during which they received standard of care treatment for wound management. During the Treatment Period, subjects were assigned sequentially to Cohort 1 or Cohort 2: Cohort 1 received once weekly doses by 2-hour intravenous infusion of SANGUINATE. Following the completion of Cohort 1, the safety findings were reviewed prior to initiating Cohort 2. Cohort 2 received 6 once-weekly infusions. In addition to the study drug, subjects continued to receive standard of care during the Treatment Period. One week after the end of Treatment, subjects completed the Venous Clinical Severity Score (VSCS), a validated instrument used to assess venous ulcer severity. The VSCS is used to determine if ulcers are healed or not at the end of Treatment. The VSCS is used to determine if ulcers are healed or not at the end of Treatment. The VSCS is used to determine if ulcers are healed or not at the end of Treatment. If ulcers were not healed at the end of Treatment, the VSCS is used to determine if ulcers are healed or not at the end of Treatment.

Results: The administration of once-weekly infusions of PEG-COHb was well tolerated. Treatment emergent adverse events (mild pyrexia, moderate wors-
en ing anemia) considered related to study drug were reported in 2/10 patients. Increased pressure were anticipated due to the oncotic effects of this colloidal drug, but with no consistent pattern to the changes. Changes in ECG intervals were seen in a few subjects, but those changes were not considered clinically meaningful. There were no clinically meaningful changes in laboratory values, physical examinations, or concomitant medications. There were no statistically significant changes from Baseline in leg ulcer pain and wound surface area for either Cohort. All of the wound assessments remained relatively consistent throughout the study. There were slight decreases in total VCSS at most time points, indicating slight improvement in vascular status. Results were similar for the individual scores.

Summary/Conclusions: The administration of 4 or 6 once-weekly infusions of PEG-COHb at a dose of 320mg/kg was generally well tolerated. Slight improvements in total and individual VCSS are promising and may warrant further study with prolonged repeated doses of PEG-COHb.

P619
NON-Renal DETERMINANTS OF ENDOGENOUS ERYTHROPOIETIN LEVELS IN SICKLE CELL DISEASE
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1Haaematological Medicine, King’s College Hospital, 2Molecular Haematology, King’s College London, 3Renal Medicine, King’s College Hospital, 4Renal Medicine, King’s College London, London, United Kingdom

Background: Sickle cell disease (SCD) is characterized by chronic hemolysis and inflammation. Elevated levels of erythropoietin (EPO) drive expansion of erythropoiesis to compensate for increased red cell destruction. EPO is produced in response to anemia and tissue hypoxia. Previous studies in SCD suggest that EPO is inappropriately low for the degree of anemia but the reasons are unclear.

Aims: To perform a retrospective analysis of data collected as part of routine clinical care to examine the relationship between serum EPO and degree of anemia, inflammation and alpha globin status, CRP and HbF. Our findings suggest negatively correlated with Hb levels, in our SCD cohort we have found only

Methods: Aims: To perform a retrospective analysis of data collected as part of routine clinical care to examine the relationship between serum EPO and degree of anemia, inflammation and alpha globin status, CRP and HbF. Our findings suggest negatively correlated with Hb levels, in our SCD cohort we have found only

Background: Sickle cell disease (SCD) is caused by polymerization of Hemoglobin S (Hbs), resulting in hemolysis and vaso-occlusion. Currently, no therapy achieving pancellular, direct inhibition of Hbs polymerization is available for adults or children with SCD. GBT440 is a novel small molecular inhibitor which increases hemoglobin oxygen affinity, thereby preventing Hbs polymerization and red blood cell sickling. This study represents the first evaluation of GBT440 in a pediatric population.

Aims: This study was designed to evaluate the safety and PK of GBT440 following a single and multiple doses in adolescents. In addition a population PK (PPK) model, based on data derived following single doses of GBT440, was developed to support the identification of future GBT440 dosing regimens for pediatric populations with SCD.

Methods: This is an ongoing, open-label, Phase 2a study in adolescents (12 to 17 years) with SCD (HbSS or HbSβthalassemia). Participants were enrolled after obtaining written informed consent/assent. This study is being conducted in 2 parts: Part A, single-dose, and Part B, multiple-dose for 24 weeks. The primary objective of Part A is PK and the primary objectives of Part B are safety and efficacy. PK samples to measure whole blood and plasma GBT440 concentrations were collected up to 15 days following single dose administration. Separate PPK models were developed to describe the concentration versus time profiles of GBT440 in whole blood and plasma using non-linear mixed effects modeling (NONMEM, version 7.3). The allometrically scaled adolescent PPK model was also used to estimate the appropriate single dose for subsequent evaluation in pediatric participants (6 to 12 years).

Results: Part A has been completed; 7 adolescents (3 males/4 females) received a single dose of GBT440 600mg. The median age of participants was 16 years (range 14 to 16 years) and the mean weight was 52.6 kg (range 44.6 to 65.8kg). GBT440 was well tolerated; there were no drug-related ≥Grade 3 adverse events (AE) or serious adverse events and the most common AE was Grade 1 nausea. A 2-compartment model with first order absorption best described the PK of GBT440 and is the same model structure as previously used for adults with SCD. GBT440 PK parameters (Table 1) are comparable to those derived in adults, suggesting that GBT440 PK in adolescents and adults are similar. Model validation confirmed this result with good agreement between the observed adolescent PK data and simulated profiles based on the adult GBT440 PK model.

Table 1.

<table>
<thead>
<tr>
<th>PK Parameter</th>
<th>GBT440 in Whole Blood (n=7)</th>
<th>Adult GBT440 (n=120)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax</td>
<td>300 μg/L</td>
<td>500 μg/L</td>
</tr>
<tr>
<td>T1/2</td>
<td>5 hours</td>
<td>7 hours</td>
</tr>
</tbody>
</table>

| Summary/Conclusions: This is the first study used to develop a GBT440 PPK model in adolescent participants with SCD. Data suggests that similar GBT440 doses can be used in adolescents and adults. Part B has been initiated to evaluate multiple doses of GBT440 in adolescents. This PPK model can potentially be used to estimate individual PK parameters (e.g., AUC) to support future GBT440 dose selection for evaluation in the pediatric population.
**P621**

DEVELOPMENT OF TAX-REDIRECTED T-CELL IMMUNOTHERAPY FOR ADULT T CELL LEUKEMIA

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**Background:** Adult T cell leukemia/lymphoma (ATL) is an aggressive peripheral T-cell neoplasm caused by HTLV-1 virus infection and its prognosis remains very poor. Tax, which is the most important regulatory protein of HTLV-1, is associated with aggressive proliferation of host cells and is also a biomarker for CD8+ cytotoxic T-cells (CTLs). We previously analyzed the Tax-specific T-cell receptor (TCR) repertoire, phenotypes and functions of Tax-specific CTLs at the single-cell level in HLA-A24+ ATL patients who underwent allogeneic stem cell transplantation (allo-SCT). We found that a particular amino acid motif (PDR) in the CDR3 region of TCR-β was conserved in different patients and also within the same patient before and after allo-SCT, and the PDR+ Tax-specific CTL clone selectively expanded in ATL long-term survivors as less-differentiated effector memory CTLs. Actually, the PDR+ CTL showed not only strong binding activity for the Tax-ligand but also strong killing activity against patients’ HTLV-1-infected T-cells without any reaction against normal cells.

**Aims:** Currently, we are planning a redirected T-cell immunotherapy using the PDR+ TCR genes for ATL. Therefore, we prepared donor-derived PDR+ TCR-transduced T-cells and evaluated their cytotoxic efficiency against HTLV-1-infected T-cells and ATL-cells both in vitro and in vivo mouse model.

**Methods:** HLA-A24-02 restricted and Tax301-309-specific TCR-αβ or γδ genes were cloned from an established PDR+ CTL clone and integrated into a retroviral vector (Tax-siTCR vector) encoding small-interfering RNAs (siRNAs) to knockdown endogenous TCR genes for the efficient expression of therapeutic TCRs. Then, CD8+ T-cells of healthy volunteers were transfected with Tax-siTCR vector (Tax-siTCLs). First, cytotoxicity and cytokine production capability of the Tax-siTCLs against HTLV-1-infected T-cells or ATL-cells were evaluated using calcein-AM-based assay and flow-cytometric analysis, respectively. Next, to evaluate the in vivo anti-ATL effects by the Tax-siTCLs, the bioluminescence assay (in vivo imaging system) was performed. We generated a luciferase-gene transduced HLA-A24+HTLV-1 infected cell-line, MT-2 (Luc-MT-2), and injected 1×106Luc-MT-2 cells into six-week-old NOD/Shi-scid IL-2RγKO Jic (NGS) mice intraperitoneally. After 3 weeks, 2×106Tax-siTCLs were administered intraperitoneally, for a total of 3×106 non-integrated Tax-siTCLs (Mock) were administered in the same way. These mice were monitored for tumor growth using IVIS system weekly.

**Results:** Tax-siTCLs showed specific and strong killing activity against both HTLV-1 infected T-cells and patients’ ATL-cells without any reaction against control normal-cells. In addition, Tax-siTCLs produced a sufficient amount of cytokines such as INF-γ, TNF-α, and IL-2 against HTLV-1 infected T-cells. In mouse experiments, the bioluminescence of Luc-MT-2 in the mice treated with Tax-siTCLs had started to reduce gradually after 7 weeks, and finally became undetectable after 9 weeks. In addition, macroscopic anatomical findings in the treated mice were normal after 12 weeks. In contrast, the amount of bioluminescence in the mice treated with Mock or in the control mice without treatment had rapidly increased and all mice died by 9 weeks.

**Summary/Conclusions:** We concluded that Tax-siTCLs could exert a strong anti-ATL effect without significant reaction against normal cells both in vitro and in vivo. The therapy using this PDR+ Tax-siTCLs has the potential to be a novel immunotherapy for ATL patients.

P622

Abstract withdrawn.

P623

NHEJ-BASED GENE EDITING: A NOVEL GENE THERAPY APPROACH IN FANCONI ANEMIA HEMATOPOIETIC STEM AND PROGENITOR CELLS

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**Background:** Allogenic transplantation of hematopoietic stem and progenitor cells (HSPCs) is the only current curative treatment for the bone marrow failure of patients with Fanconia Anemia (FA). However, the risks of GVHD and increased incidence of subsequent cancer, and the limited availability of matched donors hamper the implementation of this therapy in FA patients. For this reason correction of patients’ HSPCs by gene editing is considered a promising therapeutic alternative for these patients. In this context, gene editing constitutes a new step in the development of safe gene therapy approaches. Since non-homologous end joining (NHEJ) is the preferred DNA repair mechanism in HSPCs, and given that this mechanism has a weaker ability to generate insertions and deletions (INDELs) than homologous recombination, it has been the focus of much attention in the design of efficient gene editing strategies.

**Methods:** We investigated the efficiency of a NHEJ-mediated gene editing approach to generate compensatory mutations that can restore the FANCA protein function in HSPCs from FA patients, mimicking reversions observed in mosaic patients.

**Aims:** To demonstrate the feasibility of using a NHEJ-based gene editing strategy to correct FA-A HSPCs as a result of the insertions and deletions (INDELs) generated in edited FANCA sequences in these cells.

**Methods:** Two different FANCA mutations from FA-A patient-derived lymphoblastic cell lines (LCLs) and primary HSPCs were targeted by the CRISPR/Cas9 system. INDELs generated in a consequence of the NHEJ repair were analyzed at different time points.

**Results:** Initial studies conducted in a FA-A LCLs carrying the biallelic c.295C>T point mutation that generates a premature stop codon (p.Q99X) showed targeting efficiencies around 20%. Next Generation Sequencing (NGS) not only revealed the generation of frame-restoring repair events, but also that these frameshifted INDELs had been extended over several nucleotides, up to 50-fold expansion of corrected cells after nine days in culture, confirming the functionality and proliferative advantages conferred by the frame restored alleles.

**Summary/Conclusions:** Our results demonstrate for the first time that NHEJ gene correction is feasible in FA HSPCs. The high efficacy of the NHEJ repair pathway in HSPCs together with the simplicity of the strategy, make this approach clinically relevant for the future treatment of the hematopoietic defects in FA patients.
phoma patients. The manufacturing process consistently allows high CAR transduction efficiencies of CD3+ T cells (75.3%±4.29% and 76.95%±14.76 respectively, n=8) and ensures the preservation of CD4−/CD8− cells, which have a higher cytotoxic potential and anti-tumour activity. In vitro validation, using singly- or dual-positive CD19 and CD19 targets, demonstrated that CARiNKT19 cells are CD19-specific, retain their natural CD19 expression, and exert additive specific cytotoxicity against CD19+ and CD19+ targets. Additional functional dissection showed that activated CARiNKT19 cells, both fresh and cryopreserved, have the ability to produce cytotoxic granules and IFNγ faster and in larger amounts than same donor activated CART19 cells. Likewise, CAR2- and CAR3-iNKT cells are equally or more effective than their CART counterparts in killing CD19+ and lymphocyte cell lines (B-lymphoblastoid 1RCD1 and lymphoma-derived Fargee cells) and consistently more effective against primary MCL, MZL and CLL cells. Finally, in an in vivo NSG xenograft model of lymphoma, while survival of T- and NK cell-treated animals was the same as that of untreated animals (P=0.23), both CART19 and CARiNKT19 cell-treated animals had significantly and comparably improved overall survival (P<0.001). However, compared to CART19, CARiNKT19 immunotherapy led to a better disease control, with earlier, more profound and sustained responses resulting in a significantly improved tumour free-survival (P<0.03).

Surface expression of CARs: In our pre-clinical in vitro and in vivo lymphoma model, CARiNKT19 cells are more effective than CART19 cells against CD19+ and CD19+ B cell malignancies. Further, dual targeting by CARiNKT19 cells may mitigate against CD19-focused tumour escape after CAR immunotherapy, while the previously demonstrated role of donor iNKT cells in protection from gVHD supports the development of CARiNKT19 cells for ‘off-the-shelf’ use.

P625 A NOVEL CHIMERIC ANTIGEN RECEPTOR ENDOWS T CELLS WITH NK CELL-LIKE SPECIFICITY AND ATTACKS A WIDE RANGE OF HEMATOLOGICAL MALIGNANCIES AND CANCERS
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Background: Engineered T-cells expressing CD19-specific chimeric antigen receptors (CARs) have shown high response rates against relapsed and refractory B cell acute lymphoid leukemia (ALL). However, similar success has not yet been demonstrated in solid tumors, and the reasons for this are currently being investigated. One major obstacle is the difficulty in determining appropriate surface antigens that are effectively targeted by CAR-transduced immune cells. NKp44 is an activating receptor on human NK cells that is only expressed when the NK cells are activated, and which confers a marked increase in cytotoxicity against various tumors. Ligands for NKp44 have been reported to be expressed in various types of cancers, but not in healthy cells. Effective use of the ligand-binding domain of this receptor as an antigen recognition site of a CAR would thus allow a wide range of cancer cells to be attacked.

Aims: To determine the optimal CAR construct including the NKp44 immunoglobulin domain as a ligand-binding domain (NKp44-based CAR), with a view to developing effective CAR-T therapy against hematological malignancies and solid cancers.

Methods: We created several NKp44-based CAR constructs. Human T cells from healthy donors were stimulated with anti-CD3/CD28 beads and recombiant interleukin-2. Human NK cells were stimulated using K562-mb15-41BBL feeder cells, as previously reported (Imai C, 2005). Activated T cells or NK cells were then subjected to retroviral transduction with the CAR gene and the phenotypic and functional characteristics of CAR-T cells engrafted with the various NKp44-based CARs were compared. We determined if NKp44-ligands were present on the cell surface of various types of malignant cell lines using recombinant human NKp44 Fc chimeric protein. Results: Expression of ligands for NKp44 was confirmed in a wide range of tumor cell lines including acute myeloid leukemia (AML: KG-1, THP-1, U937, K562, Kasumi-1, Kasumi-6), T-cell ALL (MOLT-4, HSB2, Peer, Jurkat), B-cell ALL (OP-1), Burkitt’s lymphoma (Raji), neuroblastoma (NB1, NB16, IMR-32, SK-N-SH). Different expression levels of CARs were observed among the NKp44-based CARs created in this study, in which the major CAR domains, except for the ligand-binding domain, were derived from various components including NKp44, CD8α, CD28, or CD3ζ. A combination of the hinge domain from NKp44, transmembrane domain from CD8α, and intracellular domain from CD3ζ yielded the highest surface expression of CAR on both T cells and NK cells. T cells transduced with this CAR showed enhanced cytotoxicity against various target cells including AML, T-cell ALL, and B-cell ALL, but did not attack normal T cells. CAR-T cells also showed increased production of interferon-gamma and granzyme B. The hinge domain from NKp44 significantly reduced cytotoxic function, though CAR expression levels remained similar.

Summary/Conclusions: T cells transduced with NKp44-based CARs show enhanced cytotoxicity against various tumor cells. The extracellular hinge region of NKp44 appears to play an important role in ligand binding and/or recognition. NKp44-based CARs may represent a promising candidate for novel immune therapies targeting a wide range of cancers.

P626 NKP30-CAR REDIRECTED HUMAN T LYMPHOCYTES INDUCE POTENT ANTITUMOR IMMUNITY TO LEUKEMIA CELL LINES AND PATIENT-DERIVED ACUTE MYELOID LEUKEMIA IN NSG XENOGRAFT MODELS
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Background: Adoptive cellular therapy (ACT) of chimeric antigen receptor (CAR)-redirected T cells has evolved as a highly effective individualized immunotherapy for leukemia and solid cancer. In particular, clinical trials using CD19 expressing T lymphocytes to combat CD19+ lymphomas have revealed compelling results. However, suitable antigens for an effective and specific CAR-mediated therapy to acute myeloid leukemia (AML) are still warranted as e.g. CD33 and CD123 CAR expressing T cells induce potent immune reponses not only to AML blasts but also recognize normal hematopoietic stem cells (HSC). In contrast, B7H6, a member of the B7 family, is frequently expressed on various tumor cells including AML blasts while not detectable on normal tissues, and is recognized by the natural killer (NK) cell activating receptor NKp30. Moreover, NKp30 recognizes human leukocyte antigens (HLA)-B-associated transcritpt 3, a nuclear factor that is secreted and translocated to the cell surface in stressed and transformed cells.

Aims: In the current study, we thus explored the use of human T cells redirected to express a NKp30-CAR for inducing effective antileukemic immunity in vitro and in vivo to leukemia to the lymphoma clone K562 and primary AML blasts in NSG xenograft mouse models following ACT.

Methods: PBMCs or MACS® purified human T cells were polyclonally stimulated and reprogrammed with a CAR composed of the extracellular region of the NKp30 receptor fused to the CD3ζ chain signaling domain (kindly provided by Prof. Dr. H. Mack, Dept. of Internal Medicine 3, Medical University Regensburg, Germany) by retroviral gene transfer. Transduced T cells were further selectively expanded utilizing puromycin resistance present on the retroviral backbone, and NKp30 expression was determined by flow cytometry. IFN-γ ELISPOT analyses and cytotoxicity assays were performed to assess antileukemic responses to leukemia lines and primary AML blasts in vitro and in vivo using NSG xenografts and adoptive transfer of redirected T cells. Expression of B7H6 in target cells was confirmed by RNA-based RT PCR.

Results: Following transduction and puromycin selection ≥90% of CD3+ T cells expressed the NKp30 CAR. In addition, most T cells displayed an effector-memnory phenotype. Upon allo-coculture with the B7H6 expressing targets such as K562 and HL-60 (myelogenous leukemia cell lines), NALM 16 (pre-B-ALL) and patient-derived AML samples (e.g. M2506 and M2987) NKp30-redirected T cells elicited potent IFN-γ release and exhibited cytolytic activity to both leukemia lines and primary AML blasts in vitro. These responses were specific as e.g. no reactivity to B7H6 negative myeloma line U266 was observed. We then evaluated antitumoral responses of NKp30-redirected T cells in vivo. Upon adoptive transfer of NKp30-CAR T cells into NSG mice engrafted with K562 significant reduction of tumor burden was observed. Moreover, injection of 1 - 5x10⁶ HLA-A2+ CD19+ CD33+ AML-CD19+ CAR-mouse T cells into NSG mice showing up to 5% engraftment of patient derived AML blasts and thus resembling a clinically relevant minimal residual disease status at time of ACT resulted in clear leukemia regression. Further experiments e.g. to elaborate to what extent CD4+ and CD8+ T cells contribute to this antileukemic immunity are in progress.

Summary/Conclusions: These studies demonstrate that human T lymphocytes can be successfully redirected to acute leukemia by NK cell activating receptor based CARs such as the NKp30-CAR. As its ligand B7H6 has not been reported to be expressed on CD34+ HSC, this antigen might be an interesting target for adoptive immunotherapy to AML.
impressive therapeutic results recently obtained with checkpoint inhibitors have opened a new era in the field of cancer immunotherapy. Yet, clinical responses are still often observed either transiently or in a minority of patients. This underscores the need for an improved understanding of underlying factors limiting the efficacy of T cell-based immunotherapy and its wide application.

Aims: We explored an immunotherapeutic combination strategy to unleash the full activity of adoptively transferred antigen-specific T cells. We also propose to target multiple myeloma (MM) tumor cells in our established xenograft in vivo adoptive cell therapy model by T cells equipped with two optimized TCRs specific for HLA-A2.1-restricted MDM2 and P53 epitopes in combination with checkpoint inhibitors.

Methods: Human T cells from healthy donors were retrovirally transduced with MDM2- and P53-specific TCRs and expression levels were analyzed by flow cytometry. MDM2 and P53 protein expression in MM cell lines was determined by Western blot. The therapeutic efficacy of adoptive TCR transfer was evaluated in NOD-scid IL2R gamma chain (NSG) mice engrafted with L5258 cells. In these experimental conditions, NKG2D expression was strongly up-regulated under the influence of α-GalCer loaded, irradiated autologous DC in the presence of low antigenic heterogeneity and advanced differentiated effector functions of ex vivo expanded redirected lymphocytes against various MM cell lines. Furthermore, by using a sample collected from a patient relapsed with CD19 negative disease, we demonstrated the ability of the INVsh.CAR to lysate CD19-negative blasts.

Summary/Conclusions: Taken together, these findings make this receptor a promising and attractive target of a second line B-ALL immunotherapy in case of relapse after CD19-targeting therapies or for a double targeted approach. Being restricted to mature B cells, but absent on precursors and plasmablasts, our strategy could have an inferior toxicity concerning the emergence of B-cell aplasia observed in patients treated with anti-CD19 CAR-modified T cells.

P629

EXPLORING HUMAN TCR- AND CAR-REDIRECTED INKT CELLS FOR ADOPTIVE CELLULAR THERAPY

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Background: T cell receptor (TCR) - or chimeric antigen receptor (CAR) redirected T cells have substantially improved adoptive cellular therapy (ACT) for relapsed acute lymphoblastic leukemia (ALL) and multiple myeloma (MM) patients. Due to their potent antitumoral activity and minimal toxicity, CAR-T cells have been shown to be a promising approach for the treatment of relapsed disease. Despite the clinical benefits, the development of tumor escape mechanisms associated with down-regulation of antigen. Yet, we observed a strong up-regulation of PD-L1 expression in tumor cells in vivo and in vitro and in TILs which may limit the efficacy of antigen-specific TILs. Accordingly, in vivo ACT experiments combined with anti-PD1 inhibitor, demonstrated the synergistic therapeutic potential of this approach as compared to single agent. Yet, it does not result in complete tumor eradication suggesting that targeting one single immune checkpoint receptor is not sufficient to achieve tumor response.

Results: Adoptive transfer of dual MDM2/P53-specific TCR equipped T cells showed a superior anti-tumor response in vivo compared to single TCR treatment, demonstrating the need to target multiple MM antigens to circumvent tumor escape mechanisms associated with down-regulation of antigen. Yet, we observed a strong up-regulation of PD-L1 expression in tumor cells in vivo and in vitro and in TILs which may limit the efficacy of antigen-specific TILs. Accordingly, in vivo ACT experiments combined with anti-PD1 inhibitor, demonstrated the synergistic therapeutic potential of this approach as compared to single agent. Yet, it does not result in complete tumor eradication suggesting that targeting one single immune checkpoint receptor is not sufficient to achieve tumor response.

Summary/Conclusions: Combination checkpoint inhibitor approach has demonstrated potent therapeutic potential in our ACT experimental MM model and forms the basis for a novel multi-modal immunotherapeutic combination treatment for multiple myeloma.

P628

ENGINEERED T CELLS TOWARDS BAFF RECEPTOR: A NOVEL STRATEGY TO EFFICIENTLY TARGET B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: B-cell Acute Lymphoblastic Leukemia (B-ALL) is most common in children (80%), but it has also a peak of incidence in adult age. Immunotherapeutic approaches targeting the CD19 molecule paved the way for the treatment of relapsed and refractory lymphoblastic leukemia, which remains a major therapeutic challenge. Recently, the emergence of relapses with CD19-epitope loss in 10-30% of treated patients has been reported. This newly identified escape mechanism has been recently showed to be related to the combination of deleterious mutations and emergence of alternatively spliced RNA isoforms, as effect of selective pressure. B-cell Activating Factor (BAFF) Receptor is a transmembrane protein which is fundamental for B-cell maturation and survival. Moreover, the expression of this receptor is restricted to mature B cells and, interestingly, is not present on bone marrow B-cell precursors. Recent studies reported the over-expression of BAFF Receptor (BAFF-R) in various B-cell malignancies such as B-ALL, B lymphoma, chronic lymphocytic leukemia and myeloma. In the context of B-ALL, leukemic cells express both BAFF and BAFF-R suggesting the presence of an autocrine signalling loop. BAFF is also expressed in bone marrow microenvironment by endothelial cells which support the proliferation and the survival of primary B-ALL blasts.

Aims: In the current study, we aimed to develop a chimeric antigen receptor (CAR) or chimeric activating factor receptor (CABF) immunotherapeutic approach targeting the BAFF-R molecule.

Methods: We characterized the expression of BAFF-R in B-ALL primary samples. As immunotherapeutic approach to target BAFF-R molecule, we developed six anti-BAFF-R.CARs that differ for the inversion of the VH and VL and the length of the spacer domain have been generated. Cytokine-induced Killer (CIK) cells, engineered using an improved Sleeping Beauty (SB) transposon system, stably expressed anti-BAFF-CAR, and maintained their characteristic phenotype. Among the newly constructed CARs, the shortest VHH anti-BAFFR.CAR exerted the highest antileukemic activity towards target cells, such as NALM-6, with an in vitro killing efficiency of 65-75%, used in a double targeted approach.

Results: We also identified a specific cytokotoxic activity towards primary B-ALL blasts. In patients collected from a patient relapsed with CD19 negative disease, we demonstrated the ability of the INVsh.CAR to lysate CD19-negative blasts.

Summary/Conclusions: Taken together, these findings make this receptor a promising and attractive target of a second line T-ALL immunotherapy in case of relapse after CD19-targeting therapies or for a double targeted approach. Being restricted to mature B cells, but absent on precursors and plasmablasts, our strategy could have an inferior toxicity concerning the emergence of B-cell aplasia observed in patients treated with anti-CD19 CAR-modified T cells.

P630

SPECIFIC TARGETING OF ACUTE MYELOID LEUKEMIA BY THE USE OF ENGINEERED INKT (CYTOKINE-INDUCED KILLER) CELLS EXPRESSING THE ANTI-CD33 CHIMERIC ANTIGEN RECEPTOR (CAR)

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Background: We explored an immunotherapeutic combination strategy to unleash the full activity of adoptively transferred antigen-specific T cells. We also propose to target multiple myeloma (MM) tumor cells in our established xenograft in vivo adoptive cell therapy model by T cells equipped with two optimized TCRs specific for HLA-A2.1-restricted MDM2 and P53 epitopes in combination with checkpoint inhibitors.

Methods: Human T cells from healthy donors were retrovirally transduced with MDM2- and P53-specific TCRs and expression levels were analyzed by flow cytometry. MDM2 and P53 protein expression in MM cell lines was determined by Western blot. The therapeutic efficacy of adoptive TCR transfer was evaluated in NOD-scid IL2R gamma chain (NSG) mice engrafted with L5258 cells. In these experimental conditions, NKG2D expression was strongly up-regulated under the influence of α-GalCer loaded, irradiated autologous DC in the presence of low antigenic heterogeneity and advanced differentiated effector functions of ex vivo expanded redirected lymphocytes against various MM cell lines. Furthermore, by using a sample collected from a patient relapsed with CD19 negative disease, we demonstrated the ability of the INVsh.CAR to lysate CD19-negative blasts.

Summary/Conclusions: Taken together, these findings make this receptor a promising and attractive target of a second line B-ALL immunotherapy in case of relapse after CD19-targeting therapies or for a double targeted approach. Being restricted to mature B cells, but absent on precursors and plasmablasts, our strategy could have an inferior toxicity concerning the emergence of B-cell aplasia observed in patients treated with anti-CD19 CAR-modified T cells.
TREATED WITH LENTIGLOBIN GENE THERAPY

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Background: Acute Myeloid Leukemia (AML) is an aggressive malignancy still associated with high relapse rates when treated with conventional chemotherapeutic and hematopoietic transplantation regimens. In search for alternative treatment options, interest has focused on anti-sickling immunotherapies and in particular on T cells redirected with Chimeric Antigen Receptors (CARs) that have shown exciting results in cancer therapy, especially in the context of B-cell malignancies. CD33 is the only validated target in AML so far and represents a suitable antigen to be targeted with CAR-T cells, being broadly expressed on AML blasts.

Aims: The aim of the present study is to preclinically evaluate the efficacy and safety profiles of CD33 CAR redirected Cytokine Induced Killer (CIK) cells alone and in combination with standard chemotherapeutic agents.

Methods: Here we proved the feasibility of harnessing Cytokine Induced Killer (CIK) cells with a third generation anti-CD33 CAR through the non-viral Sleeping-Beauty transposon system, starting from fresh and frozen healthy mononuclear cells (PBMCs) and also from frozen primary AML samples. The in vitro anti-AML activity of CD33.CAR-CIK cells is assessed by means of cytotoxicity, proliferation and cytokine production assays upon challenge with AML cell lines and primary samples.

Results: CD33.CAR-CIK cells were able to induce a potent anti-leukemic activity as compared to unmanipulated CIK cells, in terms of specific killing (up to 70%), proliferation (up to 40% of K67+CAR-CIK cells) and cytokine production (up to 30% for both IL-2 and IFN-gamma producing CAR-CIK cells) when challenged with both AML cell lines and primary leukemic cells. By treating MAC-NRas cells grafted mice with the already established “5+3” induction chemotherapy protocol, we confirmed that chemotherapy is able to significantly reduce the leukemic burden from around 20% to 0.1% in the bone marrow. Since the AML disease is not totally eradicated, this model will be therefore suitable to further investigate the efficacy of the CD33.CAR-CIK cells immunotherapy on the chemoresistant/residual AML cells.

Summary/Conclusions: Having demonstrated the significant in vitro anti-leukemic activity of SB-modified CD33.CAR-CIK cells we next aim to assess their efficacy in vivo, particularly against the resistant/residual AML cells that were not eradicated by standard chemotherapy treatment. Moreover, envisaging a safer clinical translation of this immunotherapeutic approach, a transient CAR expression, by using CD33.CAR coding mRNA, is under investigation, in order to limit the potential myelotoxicity due to the long-term off-target effect on normal hematopoietic stem/myeloid progenitor cells. Finally, if successful, our results will provide the preclinical validation of CD33.CAR-CIK cell immunotherapy, supporting its development to the clinic.

P631

UPDATE ON THE FIRST PATIENTS WITH SEVERE HEMOGLOBINOPATHIES TREATED WITH LENTIGLOBIN GENE THERAPY

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Background: Insertion of an anti-sickling β-globin gene variant into hematopoietic stem cells (HSCs) could reduce or eliminate symptoms of severe sickle cell disease (SCD) and transfusion requirements in transfusion-dependent β-thalassemia major (TDT). LentiGlobin Drug Product (DP) contains autologous CD34+ cells transduced with the BB305 lentiviral vector, which encodes a human β-globin gene containing a single point mutation (AT87Q) designed to confer anti-sickling properties similar to γ-globin. We recently (ASH 2016) reported 23 patients with TDT, 3 have β0/βE genotypes and 1 is homozygous for a severe β+ mutation (IVS1 nt 110 G>A). Two of the β0/βE patients have completed their 2-year primary follow-up and entered a long-term follow-up study. They have been without RBC transfusions for 33 and 30 months, with total Hb of 10.9 and 13.5 g/dL, and HbAT87Q of 7.7 and 10.1 g/dL, respectively. The third patient with a β0/βE genotype has 12 months follow-up and has not required transfusions since 4 days post-LentiGlobin DP infusion, with total Hb 11.3 g/dL and HbAT87Q of 8.6 g/dL. The patient with the IVS1 genotype has 15 months of follow-up and has been free of transfusions for 11.6 months, with total Hb 8.3 g/dL and HbAT87Q of 7.8 g/dL. Since September 2016, 2 more patients with severe SCD have received LentiGlobin DP.

Summary/Conclusions: Data to date from this ongoing Phase 1/2 clinical study suggest that treatment with LentiGlobin DP elicits sustained HbAT87Q levels, which alleviate the clinical and biochemical effects of severe SCD and TDT, with safety consistent with myeloablative conditioning. Follow-up data on the 5 previously reported patients and early results from the 2 recently treated patients will be presented.
Indolent Non-Hodgkin lymphoma • Clinical

P632
A SINGLE INSTITUTIONAL EXPERIENCE OF 261 PATIENTS WITH LARGE GRANULAR LYMPHOCYTIC LEUKEMIA
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Background: Large granular lymphocytic leukemia (LGLL) is a rare clonal lymphoproliferative disorder of post-thymic T-cell or natural killer (NK)-cell lineage associated with cytopenia, splenomegaly, autoimmune disorders, and recurrent mucocutaneous infections. Treatment is dictated by the presence of these manifestations and consists of immunosuppressive therapy.

Aims: The main aim of this study is to evaluate clinical features, hematological parameters, and survival data of patients with LGLL. The secondary aim is to assess response rates and duration of response to various first line immunosuppressive therapies in LGLL.

Methods: This is a retrospective analysis of clinical and laboratory features, treatment modalities, and outcomes of LGLL patients evaluated at Moffitt Cancer Center between January 1, 1995 and May 1, 2016. Continuous and categorical variables were tested via Kruskal-Wallis ANOVA and Fisher’s Exact Test, respectively. Kaplan-Meier curves were used for overall survival (OS). P-values were two-sided with significance set at <0.05.

Results: We identified 261 patients with LGLL (91.6% T-cell, 8.4% NK-cell). Median age was 66 years [21-90] and M:F ratio was 1:2.1. Median follow up was 3.07 years [0-21.88]. 42.9% of LGLL patients presented with anemia, 37.1% with neutropenia, 30.7% with thrombocytopenia, 29.1% with bicytopenia and 6.9% with pancytopenia. Transfusion dependency was noted in 20.3%, splenomegaly in 27.3% and bone marrow involvement in 69.3%. 24.9% had autoimmune diseases and 9.2% had autoimmune cytopenias. 45.6% were observed while the remainder required at least one line of therapy. 5-year and 10-year OS were 75.0% and 63.1%, respectively. There was no statistically significant difference in OS, complete response rate or duration of response based on first line agent (methotrexate, cyclophosphamide, cyclosporine A). However, there was a statistically significant improved partial response with methotrexate versus other therapies (p=0.01). A marginally significant association between severe anemia/transfusion dependence and poor overall response rate (p=0.075) to any immunosuppressive therapy was noted. There was no statistically significant difference in OS, duration of therapy or adverse events based on absolute LGL count. Mean number of therapies was 1.08 (range 0-6) and was higher in patients with LGL count <0.5 k/µL (p=0.0078), bone marrow involvement (p<0.0001), and splenomegaly (p=0.0001).

Summary/Conclusions: In this large retrospective study, we described the frequency of LGLL-associated manifestations and their impact on the course of LGLL. Severe anemia/transfusion dependence, lower LGL counts, bone involvement, and splenomegaly were suggestive of more aggressive disease. We confirmed that there is no difference in overall survival among first line immunosuppressive therapies.

P634
ONGOING PHASE 1/2 STUDY OF INCBO50465, A SELECTIVE PI3K-DELTA INHIBITOR, FOR THE TREATMENT OF PATIENTS WITH RELAPSED/REFRACTORY B-CELL MALIGNANCIES (CITADEL-101)
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Background: Signaling networks mediated by PI3Ks have been implicated in proliferation, migration, and functioning of B-cells. INCBO50465 is a novel, potent, and selective inhibitor of PI3Kδ (≥19,000-fold more selective for PI3Kδ vs other isoforms). INCBO50465 demonstrated linear pharmacokinetics (PK) and achieved exposure levels several-fold greater than the IC90 for PI3Kδ inhibition at the recommended phase 2 dose (ASH 2016; Abstract 4195).

Aims: To evaluate INCBO50465 in patients with relapsed or refractory B-cell malignancies enrolled in an ongoing phase 1/2 study (NCT02018861).

Methods: In this phase 1/2 study, eligible patients ≥18 years of age had relapsed/refractory lymphoid B-cell malignancies (excluding Burkitt’s lymphoma and precursor B-cell lymphoblastic leukemia/lymphoma), Eastern Cooperative Oncology Group performance status score ≤2 (≤1 during dose escalation), normal liver and kidney function, and had not received autologous hematopoietic stem-cell transplant (HSCT) within 3 months or allogeneic HSCT within 6 months of screening. The protocol was initiated with a single-patient cohort, treated with oral INCBO50465 5mg QD. Subsequent cohorts used a 3+3 design and evaluated doses of 10–45mg QD. Based on PK/pharmacodynamics, the 20 and 30mg QD cohorts were expanded. Responses were assessed every 9 weeks using the Lugano Classification or International Working Group on Chronic Lymphocytic Lymphoma (CLL) criteria.

Results: As of the data cutoff (Nov 1, 2016), 52 patients were treated (median age 65 years, range 10–88). Baseline disease subtypes included diffuse large B-cell lymphoma (DLBCL; n=14), follicular lymphoma (FL; n=10), Hodgkin lymphoma (HL; n=9), marginal zone lymphoma (MZL; n=8), CLL (n=6), and mantle cell lymphoma (MCL; n=5). Sixty-two percent (n=32) of patients had ≥3 prior systemic regimens; 31% (n=16) had prior HSCT. Median duration of therapy was 3 months (range, 0.6–13.4): no DLTs were identified. Sixty-seven percent of patients discontinued therapy, most commonly due to disease progression (31%) and AEs (25%). Thirty-three percent of patients had dose interruption and 4% had dose reduction. Most common nonhematologic AEs (all grades: grade ≥3) were nausea (38%; 0%), diarrhea (31%; 6%), and vomiting (25%; 0%). Grade ≥3 hematologic AEs included neutropenia (21%), lymphopenia (17%), thrombocytopenia (10%), and anemia (4%). Forty percent of patients had serious AEs (SAEs), most frequently colitis, diarrhea, and hypotension (all n=3). One patient had grade 3 pneumonitis; none had Pneumocystis jirovecii pneumonia (PJP) or grade ≥2 elevated transaminase. Objective responses occurred at all doses (Table 1), except 5mg QD; 90% of the objective responses were observed at the 9-week disease assessment.

Table 1.

Summary/Conclusions: In patients with relapsed/refractory B-cell malignancies, INCBO50465 demonstrated manageable toxicities with no clinically meaningful transaminisits or PJP. Objective response rates were generally high and most responses (90%) were observed at the 9-week disease assessment. Different dosing regimens/schedules, long-term safety, and disease-specific cohorts are being evaluated.
lymphoma (IFL). Upon informed consent, patients receive 12 cycles of R2 induction (rituximab 375mg/m2 intravenous, 21 of 28 d, rituximab 375mg/m2 weekly cycle 1 [d1, 8, 15, 22], then d1 of odd cycles). Responders to induction (≥SD) are randomized: 1:1 to maintenance with either R2 or rituximab alone (18 cycles); following R2 maintenance, optional single-agent lenalidomide (10mg/d, 21 of 28 d) can be given until PD. The primary endpoint is progression-free survival (PFS).

Results: As of April 14, 2016, 106 patients with R/R FL have been enrolled, including 103 with grade 1-3a FL, 2 with tFL, and 1 unknown grade. Median age of patients with FL was 66 years (range, 41-91); most had ECOG PS of 0-1 (99%) and stage III/IV disease at study entry (80%). Patients received a median of 2 prior therapies (≥2, 30%); 103 (97%) patients had received prior rituximab-containing treatment, of which 35% were rituximab refractory (defined as best response of SD/PD to rituximab/rituximab-containing regimen or a CR/PR if <6 mo after the last rituximab dose). The most common prior regimens were rituximab alone (40%), R-CHOP/R-CHOP-like (38%), and bendamustine plus rituximab (35%). Premature discontinuation of lenalidomide occurred in 39 (37%) patients during the induction period, mainly due to AEs (n=20); the most common treatment-related AE leading to early discontinuation in the induction period was neutropenia in 8 patients. Four (4%) patients discontinued the study. Common grade 3/4 treatment-emergent AEs during induction in the FL safety population (n=104) were 27% neutropenia, 7% leukopenia, and 6% fatigue. At a median induction duration of 23 weeks (range, 0.4-51), 83 FL patients were evaluable for response with an overall response rate (ORR) of 65%; those who were not rituximab refractory had improved ORR compared to rituximab refractory patients (70% vs 55%; Table 1). The median time to response during induction was 2.8 mo. Twenty patients have completed 12 cycles of induction and 16 proceeded to maintenance (n=6 R2; n=10 rituximab alone). Enrollment is ongoing.

Table 1.

Summary/Conclusions: R2 induction therapy shows favorable activity and a tolerable safety profile in patients with advanced-stage, R/R FL. The study is ongoing to determine the effect of R2 vs rituximab maintenance in FL patients, and updated results will be presented.

P635

A DOUBLE-BLIND, RANDOMIZED PHASE 3 STUDY TO COMPARE EFFICACY AND SAFETY OF CT-P10 TO INNOVATOR RITUXIMAB IN COMBINATION WITH CVP IN PATIENTS WITH PREVIOUSLY UNTREATED ADVANCED FOLLICULAR LYMPHOMA


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Background: CT-P10 is the first biosimilar of innovator rituximab (RTX), approved for all indications by the European Medicines Agency. CT-P10 has demonstrated pharmacokinetics (PK) and efficacy equivalence in patients with rheumatoid arthritis (Yoo, ACR 2016) and PK equivalence in patients with advanced follicular lymphoma (AFL) (Coiffier, ASH 2016).

Aims: This study aimed to demonstrate non-inferiority (NI) of efficacy and PK equivalence between CT-P10 and RTX in patients with newly diagnosed advanced follicular lymphoma (AFL) (NCT02162771).

Methods: A total of 140 patients were randomized in a 1:1 ratio to receive CT-P10 or RTX (375mg/m2 intravenous) plus CVP (cyclophosphamide, vincristine, and prednisone) therapy every 3 weeks over 8 cycles. Overall response rate (ORR) was assessed at best overall response at cycle 6 and 8 cycles. Toxicity was assessed by the independent review committee, according to the 1999 International Working Group criteria.

Results: Therapeutic NI of CT-P10 to RTX has been demonstrated in terms of ORR over 8 cycles (Table 1). The ORR difference between two treatment groups was 4.3% in per-protocol (PP) population and 5.7% in intent-to-treat (ITT) population. Considering the statistical Non-Inferiority test using confidence interval (CI) approach with the exact binomial CI for the difference of ORR between two treatment groups, the lower bound of 95% CI lies on the positive side of -7% NI margin (-4.25% in PP population and -3.41% in ITT population).

The pre-defined non-inferiority criterion has been met with the descriptive point estimate difference approach and the formal statistical NI test with a 5% significance level. Median number of B-cells decreased to the lower limit of quantification (LLOQ) after the 1st infusion and remained at the LLOQ over 8 cycles in both groups. Overall safety profile of CT-P10 was consistent with that of RTX, including 103 with grade 1-3a FL, 2 with tFL, and 1 unknown grade. Median time to next treatment (TTNT) was applied as a new relevant measure of survival in both groups. Overall safety profile of CT-P10 was comparable to that of RTX over 8 cycles in induction period.

Table 2. Summary of Efficacy [Number (%) of patients].

Summary/Conclusions: This study demonstrates therapeutic non-inferiority of CT-P10 to RTX with combined CVP therapy in previously untreated AFL. CT-P10 was well-tolerated and the safety profile including immunogenicity of CT-P10 was comparable to that of RTX over 8 cycles in induction period.

PP population
R2 (N=96)
RTX (N=99)
Differences
Lower bound of 95% CI
TEAE
39 (41.0)
39 (40.4)
0.0%
Serious TEAE
7 (7.3)
7 (7.1)
0.0%
Death related to AE
2 (2.1)
2 (2.0)
0.0%
Infection
2 (2.1)
2 (2.0)
0.0%
* Differences was calculated using percentages not the round off values.

Table 2. Summary of Treatment-emergent adverse event (TEAE) related to the study drug [Number (%) of patients].

Safety population
CT-P10 (N=97)
RTX (N=102)
PRCT
Directly related TEAE
13 (13.5)
12 (11.8)
-7%
5.7%
Overall safety profile was consistent with that of RTX, although the proportion of patients with positive anti-drug antibody were similar between both groups (4.3% and 2.9%) over 24 weeks in the induction period.

P636

DURABLE DISEASE CONTROL OF EARLY MYCOSIS FUNGOIDES PATIENTS TREATED WITH LOW-DOSE INTERFERON-ALPHA2B AND PUVA

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Background: Early stage Mycosis Fungoides (MF) has an indolent, relapsing course, with patients frequently undergoing multiple therapies. Current guidelines consider the utility of combination therapies (skin-directed therapies plus systemic biologic response modifiers) to increase the therapeutic efficacy. Recently, time to next treatment (TTNT) was applied as a new relevant measure of the durability of response of PUVA, interferon-alpha (IFN-α) and retinoids as monotherapies in early MF (Hughes et al., Blood 2015; Hanel et al., AJH 2016), but it has not been yet investigated in combination therapies.

Aims: We aimed to evaluate TTNT together with the usual time-to-event measures (OS and EFS) in the series of 89 early MF patients treated for 14 months with interferon-α2b (IFN-α2b 6-18 MU weekly) and PUVA which was first described in 2005 (Rupoli et al., EJH 2005). The follow-up was prolonged up to October 2016, in order to evaluate prospectively the regimen activity and influence on the further course of the disease.

Methods: The design, rationale, safety and efficacy results for this protocol were previously published. Clinical stages IA-IIA patients who had received no previous treatment, or had been submitted to a 4-month wash-out after systemic therapy or a 4-week wash-out after topical therapy, were included in the study. Survival curves for each efficacy endpoint were calculated according to Kaplan-Meier.

Eighty-nine patients (56 men and 33 women) with a median age of 60 years (range, 17-80) were recruited. Disease stage was IA in 22 patients, IB in 55, IIA in 11, and IIB in 1 patient. The majority of patients had generalized skin disease (75% T2 vs 25% T1). The protocol proved to be highly effective, well tolerated and able to induce complete clearing of skin lesions in 84% of patients (55/66) over overall response rate of 98%. The median follow-up time was 175 months (range 4-259). Updated data showed that the median overall survival (OS) was not reached, whilst the median event-free survival (EFS) was 142 months (95% CI 130-153). Estimated OS rates at 1, 2, 5, 10, 15 and 20 years were
were 99%, 98%, 92%, 89%, 78% and 51%; at 1, 2, 5, 10, 15 and 20 years 98%, 97%, 88%, 67%, 19%, 0%, were free from events. Median TTNT was not reached thus indicating clinical benefit with IFN-α and PUVa. Kaplan-Meier estimated rates of 97% at 1 year, and 91% at 2 years, respectively whereas 5-, 10-, 20-year TTNT remained almost unchanged with 62% of patients that still had not required further treatment.

Summary/Conclusions: There has been an ongoing debate about whether patients would benefit from adding PUVa to IFN-α in the treatment of early stage MF. We chose to initiate the combination treatment of MF as early as possible in the course of the disease to induce a permanent remission or even a cure. In our experience, this regimen set the realistic goal of achieve high rates of complete clearing and durable responses (median TTNT not reached) with only 38% of patients requiring a subsequent systemic treatment within 20 years. Here, we suggest a synergistic or additive effect between PUVa and IFN-α compared with either agent alone. With respect to Hughes et al. (Blood 2015), our combination treatment provide a longer TTNT than PUVa or IFN-α monotherapy (36.3 months and 33.0 months respectively). At 2 years, 93% of patients receiving PUVa plus IFN-α were free from further treatment as compared to 54.2% and 29.1% treated with PUVa or IFN-α monotherapy, respectively.

P637

PHASE 3 ALCANZA STUDY OF BRENTUXIMAB VEDOTIN (BV) OR PHYSICIAN’S CHOICE (PC) OF METHOTREXATE (MTX) OR BEXAROTENE (BEX) IN CD30-POSITIVE CUTANEOUS T-CELL LYMPHOMA (CTCL): NUMBER NEEDED TO TREAT ANALYSIS

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Background: CTCL is a generally incurable, relapsing disease associated with a significant symptom burden, including disfiguring lesions, debilitating pruritus and frequent skin infections. ALCANZA is a Phase 3 study of BV vs PC (MTX or Bex) for the treatment of CD30-positive (CD30+) CTCL (NCT01578499). BV was associated with significantly improved rate of objective response lasting ≥4 months (ORR4; 56% vs 13%; p<0.0001), longer median progression-free survival (≥4 months; 16.7 vs 3.5 months; p<0.0001), and decreased symptom burden measured by Skindex-29 (27.96 vs 8.62; p<0.0001), compared with PC. BV’s safety profile was consistent with previous reports, with all-grade and grade 3 peripheral neuropathy of 67% and 9%, respectively. Number needed to treat (NNT), defined as the number of patients (pts) that need to be treated to prevent one outcome event relative to the comparator therapy, is an effective method to assess the benefit-risk of BV in a clinically relevant manner. NNT values of 3–28 have been previously reported, at various time points, in hematologic malignancies (multiple myeloma, B-cell non-Hodgkin lymphoma) to prevent one disease progression event or death. Data from the Phase 3 AETHERA study demonstrated that, at various time points, and dependent on risk group, one in every 2–4 pts treated with BV will benefit by avoiding disease progression/death. This further demonstrates BV’s clinical benefit in CD30+ CTCL pts requiring systemic therapy. This is, to our knowledge, the first report of an NNT analysis for a treatment in the CTCL setting.

Table 1. NNT analysis per IRF assessment of PFS in the ALCANZA ITT population.

<table>
<thead>
<tr>
<th>Month</th>
<th>Number of PFS events per IRF assessment</th>
<th>NNT</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3</td>
<td>7.5</td>
<td>8.44</td>
</tr>
<tr>
<td>6</td>
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<td>5.6</td>
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<td>12</td>
<td>16</td>
<td>2.06</td>
<td>1.37–3.13</td>
</tr>
<tr>
<td>24</td>
<td>37</td>
<td>2.36</td>
<td>2.16–2.58</td>
</tr>
<tr>
<td>36</td>
<td>37</td>
<td>2.26</td>
<td>2.06–2.49</td>
</tr>
<tr>
<td>42</td>
<td>43</td>
<td>1.84</td>
<td>1.57–2.16</td>
</tr>
<tr>
<td>60</td>
<td>43</td>
<td>1.67</td>
<td>1.37–2.04</td>
</tr>
</tbody>
</table>

Summary/Conclusions: ALCANZA data suggest that, at various time points, one in every 2–4 pts treated with BV will benefit by avoiding disease progression/death. This further demonstrates BV’s clinical benefit in CD30+ CTCL pts requiring systemic therapy. This is, to our knowledge, the first report of an NNT analysis for a treatment in the CTCL setting.

P638

PRIMARY OCULAR ADNEXAL LYMPHOMA OF ALL HISTOLOGIC SUBTYPES: SURVIVAL OUTCOMES AND RISK FACTORS IN LARGE COHORT OF PATIENTS AND LONG-TERM FOLLOW-UP

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Background: Although the recent reports show that interest in ocular adnexal lymphomas (OAL) and their biologic and clinical characteristics have been increased, the most OAL-related clinical study is still limited in the small number with insufficient follow-up period, result in retrospective studies with non-reproducible. Moreover, because the majority of OAL were in the low-grade histologic subtypes as primary ocular adnexal MALT (mucosa-associated lymphoid tissue ) lymphoma, there is few comparative analysis study of all histologic subtypes in OAL patients especially for non-MALT type OAL in large cohort OAL.

Aims: So our purposes of this study were to identify a correlation between histopathological diagnosis and significant parameters associated with clinical outcomes of patients with OAL in patients with diverse histologic subtypes.

Methods: We evaluated the consecutive 207 primary OAL patients who diagnosed at Catholic University Lymphoma Group (CULG) of Catholic Bone Marrow Center, Seoul between January 2004 to April 2015. Clinical information and parameters were gathered from the electronic medical records such as geographic status, complete blood count (CBC) with blood chemistry, the status of BM involvement, primary therapeutic modalities, response to initial therapy, and treatment-related complications with survival outcomes.

Figure 1. Subgroup analysis of survival outcomes according to histologic subtypes: survival outcomes in the CULG cohort. Among patients with MALT-lymphoma, there was no significant difference in OS and PFS according to therapy (Po0.05).

Figure 2. Survival outcomes in subgroups according to therapy in patients with non-MALT-lymphoma. Patients with MALT type had better OS and PFS compared with patients with non-MALT type (Po0.05).

Results: In OAL of all histologic subtypes, 10-year lymphoma-specific OS and PFS were 89.3% and 71.0% respectively. 182 patients achieved CR (87.9%). CR rate according to primary therapy was 90.4% (n=103) in T1N0M0, 95.2% (n=40) in T2N0M0, 100% (n=7) in T3N0M0, 83.3% (n=5) in T4N0M0, and 71.1% (n=27) in TxN1-4M0. Multivariate analysis in OAL of all histologic subtypes showed that the risk factors-associated PFS were positivity of BM involve-ment and non-MALT lymphoma subtype (hazard ratio; HR=5.98, p<0.001 and HR=2.96, p=0.025, respectively), the risk factors-related OS was only non-MALT lymphoma subtype (HR=9.18, p=0.013). Then, subgroup analysis

haematologica | 2017; 102(s2) | 261

Madrid, Spain, June 22 – 25, 2017
according to histopathologic subtypes, BM involvement alone was regarded as a statistically significant factor in the group of non-MALT lymphoma (HR=1.99, p=0.013) and there were no statistically significant factors in the group of non-MALT lymphoma. Although there were no risk factors with statistical significance, the BM involvement and advanced TNM stage showed a trend toward statistical significance about affecting to the failure of PFS (BM involvement of HR 5.19, p=0.054 and advanced TNM stage of HR 3.06, p=0.056). The median time-to-progression (TTP) was from 3 to 3.5 years after initial therapy in relapse or dead patients (range from 4.6 to 109.6 months).

Summary/Conclusions: Our study confirmed that OAL of all histologic subtypes also represented the indolent nature and localized behavior with favorable survival outcomes. Although BM involved OAL consisted of a small number, it was associated with poor survival outcomes. Also, relapse and lymphoma-related mortality had long-term delayed TTP, so we suggested that BM biopsy might be a necessary study for initial staging at least in all OAL and long-term follow-up is required for patients with all histologic type of OAL.

P639

CLONAL B-CELL LYMPHOCYTOSIS OF MARGINAL ZONE ORIGIN (CBL-MZ): A PROSPECTIVE REGISTRATIONAL STUDY ON 96 CASES

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Background: Clonal B-cell lymphocytosis of marginal zone origin (CBL-MZ) has been recognized as a provisional entity in the WHO classification. Despite diagnostic similarities with SMZL, the exact relation between them has not been established yet. AIM: To describe the main features of CBL-MZ and to further elucidate its relation to SMZL.

Aims: To describe the main features of CBL-MZ and to further elucidate its relation to SMZL.

Methods: 96 CBL-MZ were analyzed. Staging at diagnosis included CBCs, blood morphology and immunophenotype, biochemistry, viral test for hepatitis C and B, serum immunoglobulin levels and immunofixation as well as whole body CT scan. BM biopsies were available in 78 cases which were studied with the following panel of moAbs: CD20, DBA44, CD23, CD5, CD25, CD38, CD27, s/clgM/D, TCL-1, MNDA, T-bet and IRTA-1. Gastroscopy with multiple C and B, serum immunoglobulin levels and immunofixation as well as whole blood morphology and immunophenotype, biochemistry, viral test for hepatitis C and B were performed.

Results: A synoptic presentation of the main characteristics of CBL-MZ is given in the table. The median age was 70 y without sex predilection. By definition, no case presented with cytopenia, lymphadenopathy, splenomegaly or any other organ involvement. Median ALC and clonal B-cell counts were 5098/μL and 2880/μL respectively. 47% had paraproteinemia, mainly of the IgM type. H.pylori (+) gastritis was evident in 30%. Hp eradication had no influence on the lymphocyte counts. The percentage of BM infiltration was highly variable, ranging from 10% to 85%, with an intrasinusoidal pattern in 31%. TCL-1, T-bet, IRTA-1, and MNDA were invariably negative. MYD-88 mutation was detected in 18% and was significantly associated with IgM paraproteinemia. 6 cases were lost to follow-up. At a median follow-up time of 41 months, the majority of the cases had no disease progression (90%) and 61% had stable CBCs. 20% solely an increase in ALCs and 7% an increase in paraproteinemia only, while in 2% lymphocytosis regressed. A total of 9 (10%) pts progressed and required treatment: 5/9 due to cytopenias caused by extensive BM infiltration without splenomegaly, 1 due to bulky splenomegaly, 1 due to lymphadenopathy, 1 developed autoimmune thrombocytopenia, while in one due to high IGM levels in a MYD-88(-) case. A total of 5 (6%) pts developed splenomegaly after a median time of 78 mos (48-151).

Summary/Conclusions: After a median follow-up time of 4y we demonstrated that CBL-MZ, although displaying many diagnostic similarities with SMZL, it rarely remains to it. Most cases remain to stable, while few develop cytopenias due to an extensive BM infiltration. These latter cases apparently represent a distinct MZL category which requires further investigation.

P640

SAFETY OF SUBCUTANEOUS ADMINISTRATION OF RITUXIMAB DURING THE FIRST-LINE TREATMENT OF PATIENTS WITH NON-HODGKIN LYMPHOMA: THE MAREBRA STUDY

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Background: Intravenous (IV) rituximab is the mainstay of treatment for CD20+ B-cell non-Hodgkin lymphoma (NHL). A subcutaneous (SC) formulation of rituximab has been approved in Europe and other countries that reduces health-care resource burden and improves patient (pt) satisfaction and convenience compared with rituximab IV. MabRella is a global umbrella study comprising three local open-label, single-arm, Phase IIIb studies of rituximab SC, which share a core protocol and primary endpoint but have flexibility for exploratory endpoints (NCT01889069; NCT01987505; NCT02406092). Data from participating countries are pooled for predefined global analyses.

Aims: To evaluate the safety of first-line (1L) rituximab SC in follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL) with a focus on administration-related reactions (ARRs).

Methods: Eligible pts were aged 18–80 years with grade 1–3a FL/DLBCL and ECOG performance status ≤3. All pts had received ≥1 full dose of rituximab IV as 1L induction/maintenance before study entry, and were expected to receive ≥4 additional induction cycles (FL/DLBCL) or ≥6 additional maintenance cycles (FL). Informed consent was obtained. For induction, pts received rituximab SC 1400mg every cycle (14, 21 or 28 days) for 4–7 cycles, plus standard chemotherapy. FL pts undergoing maintenance treatment received single-agent rituximab SC 1400mg every 2 months for 6–12 cycles. The primary endpoint was incidence of ARRs, i.e. all adverse events (AEs) occurring within 24 hours of administration, considered related to study drug by the investigator. Secondary endpoints included grade ≥3 AEs and serious AEs (SAEs). The safety analysis included all pts who received ≥1 dose of study treatment. Safety data were not collected for rituximab IV, as pts entered the trial after switching to SC. Updated data are presented (data cut-off February 7, 2017).

Table 1.

Results: The safety population comprised 421 pts: 160 Italy; 140 Spain; 121 North Africa (Tunisia, Morocco and Algeria). Median age was 58 years (range 19–80); 49% of pts were male; 225 pts had FL and 196 had DLBCL. Of the pts with FL, 97 completed ≥1 cycle of rituximab SC induction (45 completed 7 cycles) and 204 completed ≥1 cycle of maintenance (175 completed 6 cycles;
Infectious diseases, supportive care

P642
MICAFOVIR VERSUS LIPosomal AMPHOTOcERIN B FOR EMpirical ANTI fungal THerapy in FEBRIle NuETROPenic PATiENTS wiTH HEMatOLOGICAl MAIgNANCIeS: A RANDOMIZED CONTROLLED TRIAL

Our aim was to compare micafovir (MCFG) vs liposomal amphotericin B (L-AMB) as empirical antifungal treatment for neutropenic patients with persistent fever of hematological malignancies. Methods: 138 hospitalized FN patients with persistent fever of HEM (AML 78, APL 4, ALL 13, MDS (RAEB) 7, NHL 28, MM 5, other hematological malignancy 3 cases) were randomized to each drug group (MCFG, 72; L-AMB, 66). The mean treatment duration for MCFG and L-AMB was 13.8 and 16.4 days, respectively. There were no significant differences in the demographics or baseline characteristics between the two groups. There were no significant differences in the efficacy between the two groups. The efficacy rates of MCFG and L-AMB were not significantly different (38/62 cases (52.8%) vs 26/66 cases (39.4%), p=0.115*), evaluated based on: (1) successful treatment of baseline fungal infection (3/4 cases (75.0%) vs 0/1 case (0%), p=0.170*), (2) absence of breakthrough fungal infection (65/72 cases (90.3%) vs 65/66 cases (98.5%), p=0.112*), (3) survival for ≥7 days after study drug discontinuation due to drug-related adverse events (45/72 cases (71.2%) vs 66/72 cases (91.7%), p=0.258*), (4) absence of premature study drug discontinuation due to drug-related adverse events (54/72 cases (70.8%) vs 47/66 cases (71.2%), p=0.615*), (5) resolution of fever during neutropenia (45/72 cases (62.5%) vs 33/66 cases (50.0%), p=0.258*). However, discontinuation due to drug-related adverse events occurred less frequently in the MCFG group (1/72 cases (1.4%) vs 6/66 cases (9.1%), p=0.005*). In safety evaluation, adverse events of creatinine increase and hypokalemia were less often in the MCFG group than in the L-AMB group (6/72 cases (8.3%) vs 19/66 cases (28.8%), p=0.001*), and were not significantly different. The MCFG group showed a trend towards better outcomes compared to the L-AMB group for the primary component end point according to the criteria of Walsh et al (N Engl J Med 2004; 351: 1391).

Results: At the time of enrolment, there were no significant differences in the demographics or baseline characteristics between the two groups. The mean treatment duration for MCFG and L-AMB was 13.8 and 16.4 days, respectively. There were no significant differences in the demographics or baseline characteristics between the two groups. The efficacy rates of MCFG and L-AMB were not significantly different (38/62 cases (52.8%) vs 26/66 cases (39.4%), p=0.115*), evaluated based on: (1) successful treatment of baseline fungal infection (3/4 cases (75.0%) vs 0/1 case (0%), p=0.170*), (2) absence of breakthrough fungal infection (65/72 cases (90.3%) vs 65/66 cases (98.5%), p=0.112*), (3) survival for ≥7 days after study drug discontinuation due to drug-related adverse events (45/72 cases (71.2%) vs 66/72 cases (91.7%), p=0.258*), (4) absence of premature study drug discontinuation due to drug-related adverse events (54/72 cases (70.8%) vs 47/66 cases (71.2%), p=0.615*), (5) resolution of fever during neutropenia (45/72 cases (62.5%) vs 33/66 cases (50.0%), p=0.258*). However, discontinuation due to drug-related adverse events occurred less frequently in the MCFG group (1/72 cases (1.4%) vs 6/66 cases (9.1%), p=0.005*). In safety evaluation, adverse events of creatinine increase and hypokalemia were less often in the MCFG group than in the L-AMB group (6/72 cases (8.3%) vs 19/66 cases (28.8%), p=0.001*), and were not significantly different. The MCFG group showed a trend towards better outcomes compared to the L-AMB group for the primary component end point according to the criteria of Walsh et al (N Engl J Med 2004; 351: 1391).

P643
ANTIFungal DRUGS INFLUENCE NeUTROPHIL EFFECTOR FUNCTIons IN ViTRo AND MODULATE PulMONARY DAMAge IN INViSIVE ASPERGILLOSIS

Background: Antifungal agents like azoles, echinocandins or polyenes substantially contribute to reduced morbidity and improved survival of high-risk patients in hematological therapy. However, besides their well-known antifungal activity there is a growing body of evidence for immunomodulatory side effects on different effector cells of the immune system.

Aims: We conducted a randomized, cooperative group, open-label trial comparing MCFG (150mg once daily) with L-AMB (2.5mg/kg once daily) as first-line empirical antifungal treatment for FN patients with persistent fever of HEM.

Methods: 138 hospitalized FN patients with persistent fever of HEM (AML 78, APL 4, ALL 13, MDS (RAEB) 7, NHL 28, MM 5, other hematological malignancy 3 cases) were randomized to each drug group (MCFG, 72; L-AMB, 66). The efficacy end point was a favorable overall response, as determined by a five-component end point according to the criteria of Walsh et al (N Engl J Med 2004; 351: 1391).

Results: At the time of enrolment, there were no significant differences in the demographics or baseline characteristics between the two groups. The mean treatment duration for MCFG and L-AMB was 13.8 and 16.4 days, respectively. There were no significant differences in the demographics or baseline characteristics between the two groups. The efficacy rates of MCFG and L-AMB were not significantly different (38/62 cases (52.8%) vs 26/66 cases (39.4%), p=0.115*), evaluated based on: (1) successful treatment of baseline fungal infection (3/4 cases (75.0%) vs 0/1 case (0%), p=0.170*), (2) absence of breakthrough fungal infection (65/72 cases (90.3%) vs 65/66 cases (98.5%), p=0.112*), (3) survival for ≥7 days after study drug discontinuation due to drug-related adverse events (45/72 cases (71.2%) vs 66/72 cases (91.7%), p=0.258*), (4) absence of premature study drug discontinuation due to drug-related adverse events (54/72 cases (70.8%) vs 47/66 cases (71.2%), p=0.615*), (5) resolution of fever during neutropenia (45/72 cases (62.5%) vs 33/66 cases (50.0%), p=0.258*). However, discontinuation due to drug-related adverse events occurred less frequently in the MCFG group (1/72 cases (1.4%) vs 6/66 cases (9.1%), p=0.005*). In safety evaluation, adverse events of creatinine increase and hypokalemia were less often in the MCFG group than in the L-AMB group (6/72 cases (8.3%) vs 19/66 cases (28.8%), p=0.001*), and were not significantly different. The MCFG group showed a trend towards better outcomes compared to the L-AMB group for the primary component end point according to the criteria of Walsh et al (N Engl J Med 2004; 351: 1391).

P644
REAL-WORLD EXPERIENCE WITH RITUXIMAB-FLUDARABINE (RF) AND DEXAMETHASONE, RITUXIMAB, CYCLOPHOSPHAMIDE (DRC) IN WALDENSTROM MACROGLOBULINEMIA: A RETROSPECTIVE STUDY FROM 163 PATIENTS

Monitoring should be part of physician’s practice in these WM patients. Long-term toxicities are also seen, at similar rates and second cancers including Richter transformation. IPSS scoring system is used to predict PFS and OS with good accuracy. Previous CLB had no impact on outcomes, but dose reductions (1.4%) were as effective as L-AMB, and better tolerated than L-AMB as an empirical antifungal therapy in FN patients with HEM.
**Aims**: The aim of our study is to clarify the immunomodulatory capacity of different antifungal drugs on the effector functions of polymorphonuclear neutrophils (PMN) and on the clinical course of invasive pulmonary aspergillosis (IPA).

**Methods**: Firstly, isolated PMN from healthy donors were preincubated with different antifungals in vitro. Here, we used the azoles fluconazole (FLU), voriconazole (VOR), amphotericin b (AmB), and the echinocandins caspofungin (CAS) and micafungin (MIC), and the polyenes amphotericin b (AmB) and liposomal amphotericin b (LAmB). Furthermore, PMN were simultaneously stimulated with lipopolysaccharides (LPS) or zymosan. Afterwards, PMN were analyzed by flow cytometry regarding activation, degranulation, and phagocytosis. Additionally, a dichotomous assay was used to detect reactive oxygen species (ROS). IL-8 synthesis was measured by enzyme-linked immunosorbent assay (ELISA). Secondly, a murine model was used to investigate the influence of MIC and POS on the clinical course of IPA in vivo. Therefore, mice were treated with antifungals and inoculated intratracheally with A. fumigatus conidia. Afterwards, mice were analyzed concerning fungal burden and pulmonary damage (albumin ELISA) with neutropenic animals serving as controls.

**Results**: In vitro, pretreatment with POS lead to enhanced activation (CD66L: 44% +/- 8 vs 13 +/- 2, *p*=0.05). Mean +/- SEM, p value ≤0.05 considered to be significant.

**Discussion**: In vivo, treatment with conventional AmB resulted in activation of almost all effector functions besides impaired phagocytosis (43% +/- 3 vs 59 +/- 3, LPS, *p*). In contrast, LAmB did not significantly after any antifungal treatment. POS resulted in reduced fungal burden as expected but led to reduced albumin concentration in BAL (111 ng/ml +/- 46 vs 380 +/- 31, *) indicating a decreased pulmonary damage. Significant difference influence on PMN effector functions in vitro, MIC did not affect clinical course IPA in vivo.

**Summary/Conclusions**: In vivo results can be considered as a reevaluation of the criteria for suspecting IFI.

### Table 1.

<table>
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<tr>
<th>Antifungal Prophylaxis with CD101 in Immunosuppressed Mouse Models of Candidiasis, Aspergillosis, and Pneumocystis Pneumonia (PCP)</th>
</tr>
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<tbody>
<tr>
<td><strong>V. Ong</strong>1, K. Bartz1, M. Cushion2, L. Migesi3, S.R. Lopez4, C.A. Therasse, A. Ferrario4, F. Pavesi5, C. Cattaneo1,*, C. Pagani1, V. Mancini2, P. Zappasodi3, A. Ferrario4, F. Pavesi5, G. Rossi1</td>
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<td><strong>RETE EMATOLOGICA LOMBARDA</strong></td>
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**Background**: Fungal infections continue to carry high morbidity and mortality. Disease- and treatment-related immunosuppression in patients with hematologic diseases increase the risk of opportunistic infection caused by Candida spp., Aspergillus spp., and Pneumocystis spp., and antifungal prophylaxis is an important consideration. Agents currently used for prophylaxis, voriconazole and TMP/SMX, carry safety and tolerability concerns. CD101 is a novel echinocandin in phase 2 clinical development that has demonstrated preclinical efficacy in treatment of invasive fungal infections and has pharmacokinetic attributes that enable once-weekly IV dosing and subcutaneous (SC) administration.

**Aims**: To evaluate CD101 as antifungal prophylaxis in neutropenic mouse models of candidiasis, aspergillosis, or PCP.
challenge on day -5,-3,-1 or -1. Survival was monitored for 14 days. PCP model: C3HHeN mice (10/gp) were immunosuppressed by dexamethasone (40μg/ml) in acidified drinking water and inoculated with Pneumocystis murina (intranasal- ly, 2 x 10⁵-50 μL). CD101 0.2, 2, or 20mg/kg intraperitoneal was given at the time of inoculation and 1x or 3x/wk for 6wks. TMP/SMX 50/250mg/kg/3x/wk was used as positive control. At 6wks, lungs were processed for quantification of trophic and asci (cyst) forms of P. murina.

Methods: A retrospective review of our Hospital’s Leukaemia database (IRB approved) was made for clinical characteristics and outcomes in surgically managed IPI patients diagnosed between Jan 2005 and Dec 2015. IPI was defined by EORTC/MSG 2008 criteria.

Results: Among 795 acute leukaemia patients diagnosed during this period, we found 19 patients with IPI who had undergone surgical interventions (15 primary, 4 relapse). The patients comprised 10 probable and 3 possible IPI. The details of the IPI, surgery-related complications, antimicrobial treatments and perioperative complications are summarized in Table 1. Most commonly performed surgical intervention was either open thoracotomy or video assisted thoracoscopic surgery for wedge resection or lobectomy. Nine of the 15 proven IPI patients had overall benefit from the procedure and achieved cure (5 with complete resolution of aspergilloma related massive bleeding and/or complete resolution of the IPI allowing further chemotherapy or transplantation). Of these, 7 patients were alive and well at the time of data collection and 2 had died. Among the survivors, the mean duration of the survival post-surgery was 57.9months (range 9–118.3 months). The 2 patients who died also had benefitted from the procedure and had survived for 6.5 and 47 months post-surgery but both succumbed to septic events unrelated to the IPI during subsequent chemotherapy. Of the remaining 6 patients (out of the 15 proven IPI), 3 had temporary clinical and/or radiological improvement only but succumbed 2 to 6 months post-surgery due to unrelated septic events, 2 died due to progression of the IPI, and 1 lacked information to draw any conclusions. The patient with probable IPI diagnosis during induction was able to proceed with further chemotherapy post-surgery but succumbed to CNS relapse of leukaemia 8 months later. Of the 3 patients with possible IPI, 2 were able to proceed with transplantation and 1 with chemotherapy post-stem-cell transplantation, but all the 3 patients succumbed to leukaemia and/or unrelated septic events.

Summary/Conclusions: Major surgical interventions are feasible in selected leukaemia patients with IPI. In carefully selected patients they can yield valuable information to guide anti-fungal therapy or enable therapeutic outcomes allowing patients to proceed with curative chemotherapy and stem cell transplantation.
nately, despite the general improvement in the care of patients with MM, no difference in the rate of infections could be detected in recent years.

P648

HUMAN L-FICOLIN POLYMORPHISMS CONTRIBUTE TO SUSCEPTIBILITY TO INFECTIONS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: In neutropenic patients with acute myeloid leukemia (AML) bacterial infections and sepsis are a leading cause of mortality. Several studies propose a contribution of individual single nucleotide polymorphisms (SNPs) of the innate immune system to the course of infections. Human ficolins represent receptors of the lectin pathway of the complement system especially ficolin-2 (L-ficolin) is emerging as an important component of the lectin pathway in the circulation. Ficolins share structural and functional characteristics with C1q from the classical pathway of the complement that acts with Pentraxin 3 (PTX3) that helps the innate immune system targeting pathogens like bacteria or viruses. In the context of hematopoietic stem cell transplantation polymorphisms of PTX3 have been identified as an individual risk factor for developing pulmonary aspergillosis.

Aims: We sought to investigate the impact of L-ficolin and PTX3 SNPs on the occurrence of infectious events such as sepsis and pneumonia, including invasive fungal disease (IFD), in 186 adult patients with newly diagnosed AML following anthracycline-based induction chemotherapy. In addition to our studies on membrane receptors, this work represents an important extension on soluble molecules of the innate immune system and their potential implication on infections.

Methods: Genotyping of L-ficolin and PTX3 SNPs (rs17514136, rs17549193, rs1800450, rs1840680) was performed by TaqMan assay. Multiple logistic regression analyses were applied to evaluate the association between SNPs of the polymorphisms and the occurrence of infectious events.

Results: Two L-ficolins SNPs were identified as risk factors for developing sepsis and/or pneumonia. Patients harboring rs1754136GG/TTAAG or GG (n=100 or 22) revealed a significantly higher risk for developing sepsis (odds ratio (OR): 1.88; 95% confidence interval (CI): 1.01–3.37, p=0.039) or pneumonia (OR: 2.79; 95% CI: 1.16–6.9, p=0.03). A similar risk profile could be demonstrated for patients carrying rs17549193TCTT or TT. No association was found between SNPs of the PTX3 gene and the analysed infectious events.

Summary/Conclusions: To our best knowledge, this study represents the first analysis demonstrating that polymorphisms of human L-ficolin (rs7309123, rs17549193) represent an independent risk factor of developing sepsis and/or pneumonia in patients with AML undergoing induction chemotherapy. Interestingly, no association of PTX3 SNPs and infectious events such as IFD was found in this non-transplant setting. In conclusion, a genetic risk profile based on membrane bound and soluble molecules of the innate immune system might be helpful in identifying patients prone for infectious events.

P649

PREDICTIVE FACTORS OF RESPONSE TO EPOETIN THETA IN CHEMOTHERAPY-INDUCED ANEMIA: A FRENCH MULTICENTER OBSERVATIONAL STUDY (PIVOINE)

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Background: Whereas erythropoiesis stimulating agents (ESA) are indicated in the management of chemotherapy-induced anemia (CIA), their use in clinical practice is a matter of controversy with regards to some meta-analyses, opinions and institutional guidelines and international guidelines. However, supportive care is an area of high importance in onco-hematology thus justifying to study the use of ESA in cancer patients in the real-life setting.

Aims: The PIVOINE study aims to provide a better knowledge about the use of epoetin theta for the treatment of CIA in onco-hematology in the real-life setting and to identify predictive factors of response to ESA.

Methods: A single institutional, prospective and observational study conducted with 136 oncologists or onco-hematologists on adult patients suffering from non-myeloid malignant tumors, treated with chemotherapy and initiating epoetin theta treatment according to standard medical practice.

Results: From November 2014 to October 2015, 137 evaluable patients were followed in the study (mean age 68.3 ± 11.3 years, 47.2% men). Overall, 21.8% of patients presented with hematological malignancies, 19.9% with digestive tumors, 18.2% with lung cancer and 40.1% with other solid tumors. The majority had a good performance status (75.2% ECOG 0-1). More than 90% of patients had never received ESA prior to enrolment in this study and 45.2% benefited from epoetin theta before chemotherapy. The median level at initiation of epoetin theta was initiated at a weekly dose of 20 000 IU for 76.1% of patients and 12.3% of patients benefited from dose adaptation during follow-up, mainly dose increase (90.5%). Overall, 18.5% of patients received blood transfusion during the study. Five-hundred and sixty-three patients (45.2%) achieved complete response (i.e. Hb level increased by at least 2 g/dl) within 12 week after epoetin theta initiation. According to Kaplan-Meier analysis, the probability of CR was 12.7% at 4 weeks, 35.8% at 8 weeks and 52.4% at 12 weeks. Multi- variate analysis showed that the lower the Hb level at baseline, the greater the chance of complete response (OR 0.4 IC95% [0.335,0.478]). Moreover, good performance status (ECOG 0 or 1), hematological malignancies (vs. solid tumor) and the absence of blood transfusion are independent predictive factors for complete response (OR 1.577 IC95% [1.186,2.098], OR 1.946 IC95% [1.459,2.597], OR 1.969 IC95% [1.411,2.747] respectively). Overall, only 27 patients (2%) experienced treatment-related adverse events, 2 of them (0.1%) presenting with a serious one (non fatal pulmonary embolism).

Summary/Conclusions: The PIVOINE study confirms that the response rate to epoetin theta varies considerably among patients treated similarly. This observational study conducted on a large population could help targeting the patients that could positively benefit from such treatment to prevent CIA, mainly patients with hematological malignancies, with good performance status and with low initial Hb level. The safety results confirmed the safety profile of epoetin theta.

P650

TIMING OF DEFIBROTIDE INITIATION POST-DIAGNOSIS OF HEPATIC VENO-OCLUSIVE DISEASE/SINUSOIDAL OBSTRUCTION SYNDROME AFTER PRIMARY CHEMOTHERAPY: EXPLORATORY ANALYSIS OF AN EXPANDED-ACCESS PROTOCOL

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Background: Hepatic VOD/SOS, which may be unpredictable and potentially life-threatening, is typically considered a complication of hematopoietic stem cell transplantation (HSCT); however, VOD/SOS can occur after chemotherapy Without HSCT. VOD/SOS with multi-organ dysfunction (MOD) may be associated with >80% mortality. Defibrotide is approved to treat severe hepatic VOD/SOS post-HSCT in the European Union and to treat hepatic VOD/SOS with renal or pulmonary dysfunction post-HSCT in the United States.

Aims: To perform an exploratory post hoc analysis of the impact of timing of initiation of defibrotide after VOD/SOS diagnosis in patients developing VOD/SOS after primary chemotherapy without HSCT (off label).

Methods: In an expanded-access protocol for patients with VOD/SOS post- HSCT or chemotherapy, with or without MOD (renal and/or pulmonary dysfunction), defibrotide 25mg/kg/d (4 divided doses of 6.25mg/kg) was given a recommended ≥21 days after patients provided informed consent. In this post-chemotherapy subgroup, survival was analyzed post hoc from the day VOD/SOS was diagnosed (days 0–30 after start of chemotherapy) through follow-up, which was collected for 100 days post-chemotherapy. For these exploratory analyses, survival rates in the post-chemotherapy subgroup were estimated. The diagnosis of VOD/SOS was defined as 2) patients starting defibrotide on a particular day: 0, 1, 2, 3, 4, 7, and 14, using Fisher’s exact test and (2) patients starting defibrotide on a particular day: 0, 1, 2, 3, 4, 5, 6, 7, 8–14, and ≥15, by Cochran-Armitage test for trend across days. Causes of treatment delay were not assessed.

Results: In the final dataset, 137 patients developed VOD/SOS after primary chemotherapy. Of these, 87 patients (41 with MOD) developed VOD/SOS by day 30 after the start of chemotherapy. In the latter group, 79.3% (69/87) were aged ≤16 years. In 26.4% (23/87) of post-chemotherapy patients, defibrotide was started the day of diagnosis; in 88.7% (79/87), by Day 7. In the population of patients provided informed consent for treatment, the median time to treatment in both the overall group and MOD subgroup (Figure), earlier initiation was associated with higher Day +100 survival rates for all days, which was significant at a number of timepoints. The trend test for particular initiation days.
also showed a significant trend over time for higher Day +100 survival with earlier defibrotide initiation post-diagnosis in both the overall group and MOD subgroup (P < 0.05). In the overall post-chemotherapy population, adverse events (AEs) and serious AEs occurred in 66% and 40% of patients, respectively. Aside from multi-organ failure, the most common AE of any severity was hypotension (9.5%). Possibly related AEs lead to discontinuation in 7.3%; most common was gastric hemorrhage (3.7%).

**Summary/Conclusions:** In this exploratory analysis of final study data in the subgroup of patients developing VOD/SOS after chemotherapy, earlier defibrotide initiation post-VOD/SOS diagnosis was associated with improved Day +100 survival, confirmed by the Cochran-Armitage test (P < 0.05), even in the small MOD subgroup. This time-dependent relationship was consistent with that found in the HSCT subgroup from this study. No specific day appears to provide a clinically meaningful cutoff for better Day +100 survival, suggesting that later intervention retains value if treatment must be delayed.

**Support:** Jazz Pharmaceuticals

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**P651**

**ADAMTS-13 REGULATES NEUTROPHIL RECRUITMENT IN A MOUSE MODEL OF INVASIVE PULMONARY ASPERGILLOSIS**

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**Background:** Von Willebrand factor (VWF) is produced as multimers of various sizes and is secreted as an acute phase protein during inflammation. The main mechanism regulating the size and prothrombotic activity of VWF is the specific proteolytic activity of ADAMTS-13 (a disintegrin and metalloprotease with ThromboSpondin type 1 repeats-13) which is diminished under several pathological conditions. 

**Aims:** To determine the relevance of this regulatory pathway for the innate inflammatory response by polymorphonuclear neutrophils (PMN), we employed a mouse model of invasive pulmonary aspergillosis (IPA) where PMN functionality is crucial for fungal clearance and survival.

**Methods:** IPA was induced by intratracheal application of *Aspergillus fumigatus* (*A. f.*) conidia in wildtype (129/Sv/Pas) or ADAMTS-13 deficient (*Adamts13-/-*) mice, and VWF deficient (*Vwf-/-*) mice or respective controls (B6). Some mice were sacrificed 24 h after infection. Fungal load was assessed as colony forming units (CFU) after plating and culturing lung homogenates on Sabouraud agar plates. For histological analysis paraffin sections of the lungs were stained with H&E, mouse complement component C3d and VWF antibody. Broncho alveolar lavage fluid (BALF) was analyzed for cell count (bead-based by flow cytometry or by an animal blood counter), ELISA was performed for albumin amount and cytokines were analyzed by a multiplex assay. Bone marrow-derived PMN were isolated by magnetic cell sorting using biotin labeled Ly6G/C specific antibody. PMN functions were analyzed for degranulation, oxidative burst activity and CD62L shedding by flow cytometry. Fungal killing of PMN in *vitro* was assessed by a XTT assay. Chemotactic properties of *A. f.*-activated and control serum from wildtype and knock-out mice was evaluated by migration of purified human PMN, isolated by dextran sedimentation and Histopaque® centrifugation, in a transwell assay.

**Results:** While infected neutropenic mice developed lethal IPA, all wildtype mice survived the infection. Interestingly, *Adams13-/-* mice displayed more severe signs of disease with a lethal course in about 24% of the animals. Examination of the lungs revealed a higher fungal burden along with increased signs of acute lung injury and levels of pro-inflammatory cytokines in ADAMTS-13 deficient mice. Histology sections demonstrated a more pronounced perivascular leukocyte infiltration in support of a dysregulated inflammatory response in *Adams13-/-* mice. Importantly, we observed no general defect in the activation of neutrophil effector functions in response to conidia or hyphae in *vitro*. Furthermore, innate inflammatory response to IPA was not altered in VWF deficient (*Vwf-/-*) mice compared to wildtype (B6) control.

**Summary/Conclusions:** Therefore, we conclude that the proteolytic regulation of VWF by ADAMTS-13 or ADAMTS-13 by itself is an important mechanism to control PMN recruitment in acute inflammatory processes, such as fungal pneumonias.
Myelodysplastic syndromes - Biology

P652
IDENTIFICATION OF THE SPECIFIC HEMATOPOIETIC STEM CELL POPULATIONS RESPONSIBLE FOR FAILURE TO HYPMETHYLATING AGENTS IN MYELODYSPLASTIC SYNDROMES
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Background: Myelodysplastic syndromes (MDS) are hematopoietic disorders characterized by the ineffective production of mature blood cells of one or more lineages and by the risk of evolution to acute myeloid leukemia. The current standard of care for MDS patients is the treatment with hypomethylating agents (HMA); however, response to drugs from this family occurs in just about half of the patients and is accompanied by high rates of therapy failure. Failure to HMA in MDS is a poorly understood process associated to increased risk of disease progression and to a dismal prognosis and cannot be, thus far, predicted or prevented.

Aims: Given that MDS are stem cell disorders, our aim was the identification and molecular characterization of the specific hematopoietic stem/progenitor cell (HSPC) population in which the relapse-driver clones arise. This is an essential step for the development of effective monitoring and early intervention protocols for HMA failure.

Methods: Using flow cytometry immunophenotyping, we quantitatively analyzed the different cell subpopulations within the CD34+CD38- and CD34+CD38+ HSPC compartments in 122 sequential MDS bone marrow samples obtained from 93 patients at different stages of HMA treatment.

Results: In line with earlier reports suggesting the presence of alterations in myeloid progenitor frequencies in MDS, our flow cytometry data stratified untreated patient samples in two groups representative of two abnormal differentiation patterns, which were independent of the IPSS risk classification. The “CMP pattern” group (12 samples, 34%) was characterized by an increased frequency of the common myeloid progenitors (CMP) (2.6-fold; p<0.05) and lymphoid-monocytic progenitors (LMP) (4.7-fold; p=0.0016), observed in CMP pattern patients but not in CMP pattern patients. HSFC frequency-monitoring of 69 samples collected from 36 patients throughout therapy showed persistence of both abnormal differentiation patterns even during clinical remission. Furthermore, specific HSC populations were differentially expanded upon HMA failure with leukemic progression in the two groups of patients. In CMP pattern MDS, LT-HSC frequency significantly increased after relapse (10.4-fold; p<0.003), whereas the LMPP frequency sharply increased (8-fold; p=10^-4) in GMP pattern patients. The fact that a proliferative switch occurred in different HSC subpopulations confirmed that the two subgroups are distinct entities with different hierarchical origins.

Summary/Conclusions: Overall, our data provide evidence of the existence of biologically different MDS subtypes which are caused by separate differentiation defects and progress through the expansion of characteristic HSC populations.

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Figure 1.

Methods: Using flow cytometry immunophenotyping, we quantitatively analyzed the different cell subpopulations within the CD34+CD38- and CD34+CD38+ HSPC compartments in 122 sequential MDS bone marrow samples obtained from 93 patients at different stages of HMA treatment.

Results: In line with earlier reports suggesting the presence of alterations in myeloid progenitor frequencies in MDS, our flow cytometry data stratified untreated patient samples in two groups representative of two abnormal differentiation patterns, which were independent of the IPSS risk classification. The “CMP pattern” group (12 samples, 34%) was characterized by an increased frequency of the common myeloid progenitors (CMP) (2.6-fold; p<0.05) and lymphoid-monocytic progenitors (LMP) (4.7-fold; p=0.0016), observed in CMP pattern patients but not in CMP pattern patients. These data suggest that each abnormal differentiation pattern arises from defects in different HSC populations and has a differential impact in the number and functionality of downstream progenitor cells (R). Agreement, a deeper immunophenotypic analysis of recently defined HPC functional fractions showed decreased erythroid and megakaryocytic potential in CMP (2-fold each, p<0.05) and megakaryocytic-erythroid progenitor (MEP) populations (3.8-fold erythroid, 7.6-fold megakaryocytic; p=0.07, p=0.04, respectively) from CMP pattern patients but not in GMP pattern patients. HSFC frequency-monitoring of 69 samples collected from 36 patients throughout therapy showed persistence of both abnormal differentiation patterns even during clinical remission. Furthermore, specific HSC populations were differentially expanded upon HMA failure with leukemic progression in the two groups of patients. In CMP pattern MDS, LT-HSC frequency significantly increased after relapse (10.4-fold; p<0.003), whereas the LMPP frequency sharply increased (8-fold; p=10^-4) in GMP pattern patients. The fact that a proliferative switch occurred in different HSC subpopulations confirmed that the two subgroups are distinct entities with different hierarchical origins.

Summary/Conclusions: Overall, our data provide evidence of the existence of biologically different MDS subtypes which are caused by separate differentiation defects and progress through the expansion of characteristic HSC populations.

Figure 1.
Background: The cytidine analog 5’-Azacitidine (AZA, Fig. A), a DNA demethylating agent, is the primary drug for the treatment of high-risk Myelodysplastic Syndrome (MDS) and Chronic Myelomonocytic Leukaemia (CMML), and response is associated with improved survival benefits. However, only ~50% of treated patients will ever respond to AZA and the molecular basis for poor response is poorly understood. It is unclear whether non-responders to therapy have different rates of AZA uptake into their cells and/or AZA incorporation into nucleic acids compared to AZA responders, nor whether these might relate to DNA methylation in vivo.

Aims: We aimed to develop an analytical method capable of simultaneously detecting all the subcellular fractions of AZA (Fig B) within the bone marrows of patients undergoing AZA therapy, while also assessing DNA and RNA methylation levels. This would provide the most comprehensive snapshot of the intracellular pharmacokinetics of AZA therapy in vivo as a first step towards better understanding AZA resistance.

Methods: We have developed a new method utilising mass spectrometry to accurately quantify all the different subcellular fractions of AZA within the same sample (Fig C). Using an Orbitrap mass spectrometer with very high mass resolution, we have achieved the first mass separation of DAC and AZA from all naturally occurring isotopes of deoxyctydine and cytidine respectively (a difference of less than 1 Da), thus enabling accurate quantification. We utilised subcellular fractionation to obtain purified quantities of DNA- and RNA-incorporated nucleotides, as well as free unincorporated nucleotides present in the cytoplasm. We developed a reaction reduction to detect the spontaneous hydrolysis of AZA and DAC, thereby greatly improving the sensitivity of detection.

Results: Using our new method, we report for the first time direct simultaneous quantification of: (1.) DNA-incorporated DAC, (2.) intracellular, free DAC, (3.) methyl deoxycytidine in DNA, (4.) RNA-incorporated AZA, (5.) intracellular, free AZA, and (6.) methyl cytidine in RNA within the same sample. We demonstrate an inverse correlation between the amount of DAC incorporated into DNA and DNA methylation. However, no such correlation was observed between AZA incorporation and RNA demethylation (Fig D). The sensitivity and resolution of our method also enabled, for the first time, a comprehensive survey of the total intracellular pharmacokinetics of AZA in vivo in patients undergoing a standard cycle of treatment.

We discovered that the bone marrow cells of AZA responders (n=4) incorporated more DAC into DNA compared to non-responders (n=4). DAC incorporation was also inversely proportional to DNA methylation levels, with greater DNA demethylation observed in the responders compared to non-responders. Furthermore, we observed two patterns in AZA non-responders, with DAC-incorporation and DNA demethylation occurring in some individuals (n=2), while in others (n=2) showed low or no DAC incorporation and no DNA demethylation (Fig E). Our method also enabled us to directly prove that low DAC incorporation was not a result insufficient AZA accumulation (n=2), while in other non-responders (n=2), DAC incorporation was also inversely proportional to DNA methylation levels, with greater DNA demethylation observed in the responders compared to non-responders. Moreover, we observed two patterns in AZA non-responders, with DAC-incorporation and DNA demethylation occurring in some individuals (n=2), while in others (n=2) showed low or no DAC incorporation and no DNA demethylation (Fig E). Our method also enabled us to directly prove that low DAC incorporation was not a result insufficient AZA accumulation in vivo.

Conclusion: We have developed a new method that has enabled the first comprehensive analysis of the intracellular pharmacokinetics of AZA therapy in vivo. Our results have revealed that while AZA responders incorporated AZA efficiently into DNA, leading to DNA demethylation, there were two modes of primary AZA resistance: in some non-responders, low levels of AZA incorporation into DNA likely derives from cell cycle quiescence, resulting in low amounts of DNA demethylation. However, in other non-responders who showed DAC incorporation into DNA and demethylation, resistance arises from as-yet unknown mechanisms not connected with AZA metabolism.

P655

CLONAL EVOLUTION OF STAG2 AND NRAS DURING PROGRESSION FROM MDS TO SAML ASSESSED BY WHOLE-EXOME AND TARGETED-DEEP SEQUENCING

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Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematological disorders at high risk of progression to acute myeloid leukemia (sAML). To date, progression dynamics and clonal evolution underlying disease progression have just begun to be understood. However, large longitudinal sequencing genomic studies are still required.

Aims: To analyze the relationship between the dynamics of gene mutations and cell pathways they are involved in with the progression from MDS to sAML in order to study the mechanisms underlying disease evolution.

Methods: Sixty-eight serially collected samples from 34 MDS/CMML patients evolving to sAML were studied by a combination of whole-exome sequencing (WES) and targeted-deep sequencing (TDS). Each patient was studied at two different time-points: at the time of diagnosis (MDS/CMML stage) and after sAML progression (disease evolution, leukemic phase). At initial presentation of the disease, diagnoses were as follows: 18 RAEB-1/2, 9 RCMD and 7 CMML. Initially, WES was carried out on 40 diagnosis/progression-matched samples. Driver mutations were identified, after variant calling by a standardized bioinformatics pipeline, by using the novel tool “Cancer Genome Interpreter” (https://www.cancergenom_interpreter.org). Secondly, in order to validate mutations and precise variant allele frequencies (VAFs) estimation, TDS using a custom MDS/AML-related capture enrichment panel (illumina) of 117 genes was performed in 30 out of 40 of the initial cohort. Moreover, a total of 28 paired samples from a cohort of 14 patients were analyzed by TDS.

Results: Combining both WES and TDS approaches, a total of 143 mutations in 50 different genes were identified at the sAML stage, with most of them (118 mutations) already present at the MDS stage, at clonal or subclonal levels. The most recurrently mutated genes were SF3B1 (41%), TET2 (41%), STAG2 (28%), SF3B1 (21%), ASXL1 (21%), TPS3 (21%) and NRAS (21%). However, it should be noted that 68% genes were mutated only in less than 10% of the patients, highlighting the great heterogeneity that exists in the mechanisms of disease evolution. To study the mutational dynamics during disease evolution we compared VAFs of mutations detected at both time-points (sAML to MDS/CMML stage) in each patient. We identified 4 different clonal dynamics: mutations that were initially present but increased VAF (type-1), decreased (type-2), were newly acquired (type-3) or persisted with similar allelic burden (type-4) at sAML stage. Interestingly, most of type-1 mutations were detected in STAG2 gene. Thus, mutational burden of STAG2 was markedly increased (6/8 patients) at sAML progression. Moreover, type-3 mutations, only detected at the sAML-stage, were predominantly identified in FLT3 (3/4) and NRAS (5/6). Conversely, type-4 mutations were present in MDS-related genes such as SF3B1 (9/12), SF3B1 (3/6) and TET2 (8/12). Most of mutations in these genes showed no changes during progression to sAML.

Summary/Conclusions: Progression from MDS to sAML could be explained by different mutational processes, as well as by the occurrence of unique and complex changes in the clonal architecture of the disease during the evolution. Mutations in genes such as STAG2, FLT3 or NRAS could play an important role during disease progression.

P656

PROGRESSION OF MDS TO AML FEATURES GAIN OF SINGLE DRIVER MUTATIONS WITH CONSEQUENT CHANGES IN CLONAL COMPOSITION, PROGRESSION OCCURRENCE OF MULTIPLE CLONES WITH MUTATIONS IN IDENTICAL GENES

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Madrid, Spain, June 22 – 25, 2017
Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic stem cell disorders with diverse phenotypes, characterized by ineffective hematopoiesis and mal functional clones harboring distinct combinations of different clonal mutations in different clones. As a consequence, there is a fertile ground (e.g. microenvironment) for such mutations in a patient and may lead to (a therapeutically exploitable) competition of clones.

Aims: Here, we assessed mutations in serial samples of patients with MDS and progression to AML by next generation (NGS) and single-cell sequencing to identify mutations and clonal changes associated with AML development.

Methods: Mononuclear cells from 21 bone marrow (BM) samples of 8 patients with MDS and progression to AML were studied for mutations. We used a hybrid capture panel for 31 genes known to be recurrent in AML patients and humanized mouse BM cell cultures (MSCs). Moreover, we developed an in vitro 2D co-culture system as an alternative/complementary tool to in vivo studies.

Results: Our data showed promising results with the injection of nucleolar cells obtained from patient BM, however the co-injection of mesenchymal stromal cells (MSCs) did not improve the level of engraftment. To address the question of the becoming of MSCs once injected, we tracked them back into the mice BM and showed that they disappeared after a week of engraftment. With a 2D vitro system, we showed that we could co-culture CD34+ cells with BM, or two populations of mesenchymal MSCs, over 4 weeks with a fold expansion ranging from 50 to 600 times. More importantly those cells conserved their clonal architecture and directional characteristics.

Results/Conclusions: The in vitro model cannot be replaced, the low level of engraftment of most of the patients is a limit in the study of MDS. Here we have demonstrated the value of the 2D co-culture system using MSCs (or murine MS5) as an alternative model to study MDS. This ex vivo culture system, which lasts for only 4 weeks and requires low number of human CD34+ cells, provides a robust preclinical assessment tool to test therapeutic effects of different drugs and other approaches on the MDS clonality and autologous MSCs prior to treatment of MDS patients.
the controls (88 μM/kg), suggesting an attempt to compensate the energy imbalance with the increment of anaerobic glycolysis. MDA level, which reflects the lipid peroxidation, is 1mM in young subjects, 9mM in elderly subjects, 9mM in b-thalassemia and 15mM in MDS. In vitro iron chelation partially restored the lipid peroxidation, is 1mM in young subjects, 9mM in elderly subjects, 9mM in b-thalassemia and 15mM in MDS. In vitro iron chelation partially restored the lipid peroxidation, is 1mM in young subjects, 9mM in elderly subjects, 9mM in b-thalassemia and 15mM in MDS.
Myelodysplastic syndromes - Clinical 2

P662

A PHASE II STUDY EVALUATING THE SAFETY AND CLINICAL ACTIVITY OF ATEZOLIZUMAB ALONE AND IN COMBINATION WITH AZACITIDINE IN PATIENTS WITH RELAPSED OR REFRACTORY MYELODYSPLASTIC SYNDROMES

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Background: Treatment options are limited and prognosis is poor for patients (pts) with relapsed or refractory myelodysplastic syndromes (R/R MDS), those who relapse after or fail to achieve complete response with hypomethylating agents (HMAs). Atezolizumab (atezo) is a humanized IgG1 monoclonal antibody that binds programmed death-ligand 1 (PD-L1), disrupting the immune activation checkpoint. The role of PD-L1 blockade in hematologic disorders is not yet established. Immune evasion via the PD-L1/PD-1 pathway may play a role in MDS resistance to HMA treatment (Yang et al. Leukemia. 2014). Upregulation of PD-L1 expression seen in pts during and after HMA treatment suggests that atezo could provide benefit in HMA-exposed pts as a single agent or in combination with an HMA.

Aims: To determine the safety and clinical activity of atezo alone and in combination with azacitadine (aza) in an open-label Phase Ib clinical trial (NCT02508870).

Methods: Pts with R/R MDS were eligible for Cohorts A and B, excluding those with prior solid organ or allogeneic hematopoietic cell transplant. Pts in Cohort A received atezo 1200mg intravenously (IV) on day 1 of each 21-day cycle for up to 17 cycles. Pts in Cohort B received aza 75mg/m² on days 1 through 7 and atezo 840mg IV on days 8 and 22 of each 28-day cycle for 6 cycles, followed by single-agent atezo 1200mg IV on day 1 of each 21-day cycle for up to 6 cycles. Primary endpoints were safety and tolerability. Secondary endpoints included overall response rate, time to AML progression, PFS, OS and changes in transfusion rate. Blood and bone marrow samples were collected for PK evaluation and biomarker analysis.

Results: At the time of data cutoff, 10 pts in Cohort A and 6 pts in Cohort B were safety evaluable. The median age was 76 years (range, 63-89 years); 4 of 16 pts (25%) had ≥2 previous lines of therapy. All pts were previously exposed to aza, and 2 of 16 pts (13%) were also exposed to decitabine. 15 of 16 pts were refractory to prior therapy. All pts experienced ≥1 treatment-emergent adverse event (AE). The most common Grade 3-4 AEs were febrile neutropenia (31%) and decrease in neutrophil count (25%). One pt died on study of an unknown cause. The median duration of treatment was 101 days (range, 70-275 days) in Cohort A and 92.5 days (range, 39-144 days) in Cohort B. Four of 10 pts in Cohort A and 3 of 6 pts in Cohort B remained on study as of 16 Sep 2016. All 10 pts in Cohort A and 5 of 6 pts in Cohort B were evaluable for response. No pt achieved an objective response; 6 of 10 pts (60%) in Cohort A and 3 of 5 pts (60%) in Cohort B had stable disease. Compared with pretreatment, a trend toward decreased RBC transfusions was observed in pts in Cohort A, especially in those who remained on therapy beyond 12 weeks. Preliminary PK analysis showed that exposure to atezo as a single agent or in combination with aza was comparable to historical data in pts with solid tumors.

Summary/Conclusions: Early evaluation of atezo alone and in combination with aza suggests that the safety profile is consistent with that expected in the study population. Additionally, the duration of therapy observed is encouraging in this R/R population with no standard-of-care options. Updated safety, efficacy and survival data will be presented. Emerging correlative biomarker information will also be discussed.

P663

EPIGENETIC DRUG TREATMENT GLOBALLY INDUCES CRYPTIC TRANSCRIPTION START SITES ENCODED IN LONG TERMINAL REPEATS (LTRS)

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Summary/Conclusions: The maturation database (using the maturation analysis from Infinicyt® software) was useful to discriminate between MDS patients and non-clonal cytopenias, proving to be a reliable diagnostic test, also with prognostic implications. The application of this database as a diagnostic tool has the advantage that the result is independent of the observer. Inclusion of more myeloid markers and incorporation of erythroid parameters could increase sensibility in differential diagnosis.
LYMPHOPENIA IS AN INDEPENDENT RISK-FACTOR IN PATIENTS WITH LOW-RISK MDS ACCORDING TO THE IPSS-R

Aims: To clarify the prognostic impact of lymphopenia in MDS in addition to the Revised International Prognostic Scoring System (IPSS-R)

Methods: The Düsseldorf MDS-registry was searched for patients with a complete differential blood count at diagnosis. Patients having received allografting or with an absolute lymphocyte count >5.0 G/l were excluded. The influence of the absolute lymphocyte count at diagnosis on overall survival was determined by the Kaplan-Meier analysis. Multivariate Cox regression analyses were performed.

Results: 2035 patients (RA n=182, RCMID n=978, RARS n=170, MDSd5q n=92, RAEB-12 n=163) with a median follow-up of 23 months (mo) were identified. Data were sufficient for IPSS-R calculation in 651 patients. The mean absolute lymphocyte count (ALC) in the whole population was 1402/μl (95% CI: 1368-1437, range 0.12-4972) with no significant differences between the IPSS-R groups (very low-risk [n=77] mean 1471/μl, low-risk [n=255] mean 1406/μl, intermediate-risk [n=154] mean 1244/μl, high-risk [n=96] mean 1419/μl, very-high-risk [n=69] mean 1255/μl, p=0.067). 688 patients (34%) were lymphopenic (ALC < 850/μl) with a significantly shorter survival (median 1078 vs 1971/μl, p=0.001) than non-lymphopenic patients. After stratification according to IPSS-R, survival of lymphopenic patients was not significantly different in the very-low, intermediate or very high risk group. Within the low risk group the survival difference was not of borderline significance (median 67 vs 47 months, Log Rank p=0.1, Breslow p=0.039). With an ALC above the first quartile of the whole population (850/μl) as discriminator, the survival difference between lymphopenic and non-lymphopenic patients within the IPSS-R low-risk group reached statistical significance (survival median 67.4 versus 43.0 months, Log Rank p=0.002). This was not the case in the other IPSS-R subgroups. In multivariable analyses, an absolute lymphocyte count < 850/μl was the strongest independent prognostic value for the IPSS-R low-risk group after inclusion into a Cox regression model together with age (>70 and LDH (< normal value (240 U/l) (p= 0.039). Patients with an ALC <850/μl had significantly lower platelet (median 97 versus 150 G/l, p=0.001) and neutrophil (median 1478 versus 1971/μl, p<0.001) counts but similar haemoglobin levels (median 12.4 versus 9.4 G/l, p=0.25).

Summary/Conclusions: An absolute lymphocyte count < 850/μl is an independent risk factor in patients with low risk MDS according to the IPSS-R. Whether lymphopenia in MDS is a direct consequence of the underlying haematopoietic stem cell defects or arises from immune-modulating stimuli due to the disease or to other host conditions remains to be elucidated. The lower levels of platelets and neutrophils in lymphopenic patients observed in our cohort point towards an association of lymphopenia with marrow insufficiency. In addition, further studies with larger patient cohorts are necessary to define the lymphocyte count most suitable for prognostication.

IMPACT OF MARROW COMPLETE RESPONSE IN THE NATURAL HISTORY OF PATIENTS WITH MEYLODYPLASTIC SYNDROMES (MDS) AND CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML) TREATED WITH HYPOMETHYLATING AGENTS

Aims: The aim of our study was to describe the impact of mCR in survival outcomes in patients with MDS treated with hypomethylating agents (HMA).

Methods: We retrospectively reviewed 713 patients diagnosed with MDS or CMML and treated with frontline HMA between 2004 and 2015 at a single institution. Clinical and demographic data were obtained from an electronic data base. Response was assessed by modified 2006 IWG criteria. Statistical analyses were performed with the IBM SPSS Statistics 23.0 software. All tests were 2-sided with significance level set at p<0.05.

Results: 26.3% patients from the initial cohort achieved at least hemato logical improvement (HI) as best response and were included in the analysis. 162 (37%) patients were female. Median age at diagnosis was 68 years (range 17-91). Following the 2016 WHO classification: 30% patients (7%) were MDS-SLD, 50 (11%) MDS-MLD, 20 (5%) MDS-RS, 230 (52%) MDS-EB, 10 (2%) MDS-U and 104 (23%) CMML. According to the Revised International Prognostic Scoring System (IPSS), 37 patients (8%) belonged to the low risk group, 176 (40%) to the intermediate-1 risk group, 198 (45%) to the intermediate-2 risk group, and 31 (7%) to the high risk group. 200 (45%) patients received azacitidine-based therapies and 244 (55%) decitabine-based therapies. Responses included: 238 (33% of the total population) complete responses (CR), 91 (9%) mCR, 2 (<1%) partial responses (PR) and 143 (20%) stable disease (SD). HI was observed in 410 (58% of the total population) of the patients. The median time to response was 3 cycles (range 1-24). Median overall survival (OS) since the...
Luspatercept increases hemoglobin and reduces transfusion burden in patients with lower-risk myelodysplastic syndromes (MDS): Long-term results from phase 2 PACE-MDS study


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Background: Management of anemia is a common therapeutic challenge in patients (pts) with MDS. Luspatercept (ACE-536), a fusion protein containing modified action receptor type II B, is being developed for treatment of anemia in lower-risk MDS. Luspatercept binds to TGF-β superfamily ligands (such as GDF11) reducing aberrant Smad2/3 signaling and promoting late-stage erythroid differentiation and increased hemoglobin (Hgb) levels (Suragani R, Nat Med, 2014; Attie K, Am J Hematol, 2014).

Aims: This ongoing, phase 2, multicenter, open-label study followed by a long-term extension (ext) study evaluates the efficacy of luspatercept in pts with lower-risk MDS. Endpoints include long-term safety and tolerability, erythroid response (IWG HgE), RBC transfusion independence (RBC-TI, ≥8 weeks), duration of HgE, pharmacodynamic and iron metabolism biomarkers, and pt-reported quality of life (QoL).

Methods: Inclusion criteria: MDS IPSS low or int-1, age ≥18 yr, Hgb <10 g/dL (if <4U RBC/8 weeks), no prior HMA, and no current lenalidomide or erythropoiesis-stimulating agent (ESA). The dose-escalation phase of the study is completed. An expansion cohort of up to 56 patients was added to this phase to evaluate response to luspatercept in pts who would not qualify for the phase 3 MEDALIST trial (for regularly transfused ring-sideroblast positive [RS(+)] patients with EPO >200 U/8 weeks). These include pts with low transfusion burden (LTB, <4U RBC/8 weeks) and either 1) RS(−) (12% in bone marrow) with baseline EPO ≤200 U/8 weeks or 2) RS(+) and any EPO level. RS(+) pts are treated with ≥0.75 mg/kg every 3 weeks for up to 5 doses (titration up to 1.75 mg/kg) in the base study (NCT01749510) and then are eligible for long-term treatment up to 5 additional years (NCT02268383).

Results: Data (as of 9/09/2016) were available for 73 base and 42 ext study pts. At week 52, 22 ext pts were LTB, 41 base pts were ≥4U RBC/8 weeks. Luspatercept demonstrated robust and sustained increases in Hgb and decreases in transfusion burden (HTB, ≥24 U RBC/8 weeks) compared with baseline (LRB, >4 U RBC/8 weeks). Median (range) EPO was 6.4 (1.6-20.8) U/8 weeks for LTB pts and 10.2 (1.7-140) U/8 weeks for high transfusion burden (HTB) pts. Preliminary RS(−) response rates (IPSS) by subgroup were 62% (18/29) and 19% (4/21) for RS(−) pts with EPO <200 U/8 weeks and ≥4% (5/11) and 8% (7/87) for RS(+) pts with EPO 200-500 U/8 weeks. RBC-TI rates for pts treated with ≥0.75 mg/kg in the base and ext studies, respectively, were 66% (4/12) and 68% (9/13) for RS(−) pts with EPO <200 U/8 weeks and 44% (2/4) and 57% (4/7) for RS(−) pts with EPO 200-500 U/8 weeks. Preliminary RS(+) response rates (IPSS Hgb and RBC-TI) by subgroup will also be presented at the meeting. Luspatercept was well tolerated, with related grade 3/4 adverse events (in 3 pts as of 23Nov2016) of blast cell count increase, myalgia, and worsening of general condition. The most common related AEs (≥2 pts) were diarrhea, fatigue, headache, hypertension, arthralgia, bone pain, injection site erythema, myalgia, and peripheral edema.

Summary/Conclusions: Lower-risk MDS patients treated long-term with luspatercept demonstrated robust and sustained increases in Hgb and decreases in transfusion burden and a high rate of RBC-TI. A Phase 3 study of luspatercept in regularly-transfused RS(+) patients with lower-risk MDS according to IPSS-R is ongoing (MEDALIST study; NCT02631070).

P667

RATe AND CAUSEs OF 5-ACeTyciDiNe DiSCONTinuAtion AND SUBLsequent THERAPeUTic OPCIOnS IN 418 MDS PATIENTS FROM THE ITALIAN MDS REGISTRY OF FONDAZIONE ITALIANA SINDROMI MIELODISPLASTICHE (FISM)


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Background: Azacytidine (AZA) is the current standard of care for patients with high-risk myelodysplastic syndrome (MDS) in Europe. AZA has shown a
survival advantage when compared with conventional therapies and has also shown activity in IPSS lower-risk patients. However, about 40% of patients do not respond and most patients lose response within 2 years. Treatment options for MDS patients failing hypomethylating agents therapy are scarce and overall survival (OS) is extremely short.

**Aims:** Objectives of this study were to describe in a cohort of real life MDS patients treated with AZA, the reasons causing treatment discontinuation, and to evaluate the clinical outcome after the end of AZA therapy.

**Methods:** Unselected patients included in the MDS Registry of Fondazione Italiana Sindrome Mielodisplastica (FISM) and treated with AZA from January 2009 to June 2014 were considered for the analysis. All types of conventional MDS were allowed. Clinical response, cause of discontinuation, salvage treatments and OS from discontinuation of AZA were the major end points.

**Results:** Between January 2009 to June 2014 1799 newly diagnosed MDS patients were enrolled in the Registry, and 418 received AZA; 269 as 1st line treatment (64%), and 149 as salvage treatment (36%) and 34 as a line ± ASCT (8%). Median age was 73 years (range 18-91); 260 patients (62%) were male. WHO diagnosis was RA or RARS (n=27, 6%), RCMD with or without RS (n=62, 15%), AEB1 (n=126, 30%), AEB2 (n=189, 45%), other subtypes (n=15, 4%). At start of AZA therapy IPSS score was low in 14 (3.4%), int-1 in 97 (23.2%), int-2 in 163 (43.8%), high in 67 patients (16%), and not available in 57 patients (13.6%). Patients received a median of 7 courses of treatment (range 1-63). Seventy-three % of the whole cohort (418 pts) were alive at 1 year from beginning of AZA therapy and median OS was 23 months. (25 for IPSS lower-risk MDS and 21 for IPSS higher risk MDS). OS after discontinuation of AZA was 8 months. Clinical responses according to IWG criteria were available in 344/418 patients (82%); 51 (15%) patients achieved a complete hematological response, 77 (22%), a partial response, 86 (25%) had stable disease while 136 (33%) did not respond. Response was achieved after a median of 6 cycles. After a median follow up of 16 months (range 7-35) in 37 (9%) patients AZA therapy was still ongoing while in 381 (91%) the treatment has been discontinued. Interruption of treatment was due to loss of response in 59 (16%) patients, AML evolution in 154 (40%), death in 43 (11%), toxicity or poor compliance in 39 (10%), allogeneic transplant (HSCT) in 12 (3%), other reasons in 22 (6%), not reported in 52 patients (14%). Of the 381 patients who discontinued AZA, 15 (4%) were managed with intensive AML-like chemotherapy, 22 (6%), received an allogeneic HSCT, 27 (7%) low-dose chemotherapy (7%), 22 (6%) erythroid stimulating agents, 18 (5%) other treatments and 277 (72%) patients no further treatment or only supportive therapy.

**Summary/Conclusions:** Our data confirm that AZA therapy is effective for MDS patients, both with higher and lower IPSS risk disease. Response rate is consistent with what previously reported, with a median OS of 23 months. Interestingly, at 16 months, 91% of patients had discontinued treatment, either for progression or loss of response and only in 10% of cases for reported toxicity. Only 28% of patients received any kind of salvage therapy and overall survival after AZA discontinuation was poor (8 months).

**P668**

**COMBINATION OF DEEP PHENOTYPING AND TARGETED NEXT GENERATION SEQUENCING AS A DIAGNOSTIC TOOL IN CHILDREN WITH SUSPECTED MDS**

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**Background:** Paediatric Myelodysplastic Syndromes (MDS) are a rare and heterogeneous group of disorders distinct from adult MDS. They may present with symptomatic anaemia, life threatening infection or evolving leukaemia; however, they may also present as unexplained cytopenias or with multisystem disease of unclear aetiology. Diagnosis can represent a huge challenge for clinicians, even in highly specialised centres and this can delay the delivery of the most appropriate treatment. Hence an accurate diagnosis is crucial in selecting the most appropriate management, including surveillance and follow up.

**Aims:** To devise a clinical grade diagnostic targeted NGS panel and combine the results with extensive clinical phenotypic information to obtain a diagnosis in children referred with suspected MDS.

**Methods:** Children (0–18yrs) were referred from 14 UK centres with a diagnosis of suspected MDS and/or sustained cytopenias with morphological features of myelodysplasia. Extensive phenotypic information including family history, detailed clinical examination and disease course details were collected and captured on an online database using the Human Phenomiser tool. A customised targeted NGS panel was designed using the illumina design studio containing 32 genes, 916 amplicons and 301 exons; selected through literature reports and well described mutations in Paediatric MDS and potential overlap Bone Marrow failure syndromes (BMFS). Coverage of each base within target regions was assessed for every sample on each sequencing run using Coveme software. Library preparation was performed using an illumina TrueSeq Custom Amplicon panel, followed by sequencing on an illumina Miseq. Data analysis was performed using our established bioinformatic pipelines (Humblin A: Blood 2014 124:2373).

**Results:** In total 59 patients (females= 29, males 30) have been screened and 3 subgroups identified based on the original suspected clinician diagnosis at presentation: MPNJMML (n= 15), de novo MDS (n=9) and idiopathic cytopenias of undetermined significance, (ICUS) with some features of dysplasia (n= 35). Mutations were detected in 24/59 patients (40%, Table 1). Of these, NGS results confirmed the original clinical diagnosis in 15 cases (62.5%); established the diagnosis for the first time in 6 cases (25%); and led to a change in diagnosis (from autoimmune neutropenia to Shwachman-Diamond Syndrome) in 1 case leading to a significant change in patient management. In two already known cases, it allowed monitoring of the disease molecular signature. As expected, BMAS pathway mutations were common in the JMML/MPN (100%) and de novo MDS patient subgroups (33%). Additional mutations in epigenetic modifiers, spliceosomes mutations as well as second BMAS pathway hits were also detected in 40% of JMML patients and in one case within the de novo MDS group; this finding was associated with poor outcome. Within the heterogenous ICUS patient group, pathogenic mutations were identified in 5/35 (14.3%) cases with BMFS genes (SBDS, ELANE, TP53). In contrast to the other MDS/MPN cases, in this group, no BMAS pathway mutations were detected.

**Table 1.**

**Summary/Conclusions:** Targeted NGS together with detailed phenotyping is a useful tool for the diagnosis of suspected MDS and unexplained cytopenias in children, with 40% of patient showing a disease-associated mutations. Results were available within 6-8 weeks in most cases enabling both rapid initial diagnosis and, in some cases, appropriate molecular markers for monitoring clonal evolution and response to therapy. For the children who remain without a clinical diagnosis, whole genome sequencing (WGS) may identify pathogenic mutations and this is currently underway.
Myeloma and other monoclonal gammopathies - Clinical 3

P669
OUTCOMES IN PATIENTS ALLOCATED TO NO-ASCT BASED ON DEPTH OF RESPONSE: INITIAL RESULTS OF A PHASE 2 TRIAL ASSESSING THE IMPACT OF MINIMAL RESIDUAL DISEASE (MRD) IN PATIENTS WITH DEEPLY RESPONSE TO RVD AND ASCT (P669)

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Background: In multiple myeloma (MM) the interactions between malignant plasma cells and the bone marrow (BM) microenvironment are important for treatment outcome. There is limited data on the effects of lenalidomide (LEN) on the BM immune profile and its therapeutic predictive value. The FMG-MM02 study (NCT01790737) was designed to explore the response to LEN, bortezomib and dexamethasone (RVD) induction, followed by a single autologous stem cell transplantation (ASCT) and LEN maintenance as a first-line therapy for MM patients (n=80). The primary endpoint was achievement of an immunophenotypic remission. Here we report the results of one of the secondary endpoints: composition of lymphocyte subsets at baseline and during LEN maintenance.

Aims: The aim of this study was to assess the proportion of different lymphocyte subsets at baseline and after ASCT and correlate lymphocyte composition with patient outcome.

Methods: Flow cytometry (FC) panel included antibodies against CD38, CD138, CD45, CD19, CD56, CD27, CD28, CD81, intracytoplasmic lambda. Sequential analyses (at baseline, after induction and at 9, 16 and 26 months after ASCT) were performed in 37/80 patients who achieved at least near complete remission (nCR) or early relapse within one year of ASCT.

Results: At baseline, markers for disease burden, such as the percentage of myeloma cells in BM or paraprotein levels did not differ between the response cohorts. No differences were noted in R-ISS or ISS risk stratification either, but there were more IMWG high risk patients in the control cohort (p=0.048). The median percentage of total lymphocytes (15.4±4.1%) and myeloma cells in BM or paraprotein levels did not differ between the response cohorts: the good cohort (n=26) defined by persistent stringent complete remission (sCR), T-cells (9.6±5.7) and CD38+ T-cells (0.9±0.7) were all higher in the good response cohort at baseline. In particular, the median proportion of mature B cells in BM was significantly higher in the best cohort at baseline (1.32 vs 0.91; p=0.02), after induction (0.27 vs 0.13; p=0.002) and 16 months after ASCT (1.73 vs 0.56; p=0.008) (Figure 1).

Summary/Conclusions: Composition of the BM lymphocyte pool at treatment baseline may have an influence on treatment outcome in multiple myeloma. More detailed subtyping of the lymphocyte phenotypes is ongoing and may reveal potential predictive biomarkers for immunomodulatory drugs such as lenalidomide and checkpoint inhibitors.

Figure 1.

Summary/Conclusions: The study was designed to evaluate a stratified approach to ASCT, investigating if patients in deep remission to induction may safely be assigned to delayed ASCT.

Aims: This single arm phase 2 clinical trial conducted at 13 UK sites aimed to determine the progression free survival (PFS) for patients who achieved ≥VGPR to induction therapy with no maintenance. Here we report the primary endpoint and stratify in the patients not proceeding to ASCT, and the influence of MRD status on PFS.

Methods: NDMM patients eligible for ASCT received PAD (bortezomib 1.3mg/m² IV or SC daily days 1, 4, and 8-11; daily days 8-11 and 15-18 for cycle 1 only) for 4-6 cycles. Those achieving <PR were off protocol; all others had PBSC collected by restaging including MRD assessment on bone marrow using multi-parametric flow cytometry. Those in PR were stratified to ASCT (no maintenance) whereas those achieving ≥VGPR stopped therapy. Responses were assessed at 100 days post PBSC collection (including MRD), and at monthly intervals for up to 2 years. High risk disease was defined by the presence of one or more adverse FISH lesions (t(4;14), t(14;16), t(14;20), del(17p13), +1q21).

Results: Between April 2011 and January 2014 153 patients were enrolled (median age 55, range 28-71 years), 139 (91%) received 4-6 cycles of PAD. The majority (88.2%) received SC bortezomib, 18 (11.8%) received at least 1 cycle IV. FISH data was available for 132 patients, 89 (67.4%) patients were ≥VGPR, 13 (10.0%) patients were ≥CR, 32 (24.2%) patients were ≥PR, 51 (38.6%) patients were ISS 1, 67 (44.1%) ISS 2 and 34 (22.4%) ISS 3. The overall response rate to PAD was 82.4% (≥VGPR: 41.2%). Responses were similar irrespective of ISS or genetic risk standard: ≥VGPR 37.5%, PR 40.9%, adverse risk; ≥VGPR 53.3%, PR 34.9%.

Post-PBSC, 63 (41.2%) patients achieved ≥VGPR, and 44 (28.8%) patients achieved PR of whom 36 proceeded to ASCT. At median follow-up of 44 months from registration, median overall PFS was 22.5m (95% CI: 18.1-25.3). For those who achieved ≥VGPR, median PFS from PBSC collection was 8.9m (95% CI: 4.6-13.3) and 25.7m (95% CI: 13.7-37.6) for MRD- (N=25) and MRD+ (N=16) patients at D100 post-PBSC respectively, 2-year PFS 28.0% (95% CI: 19.4-45.6) and 56.3% (95% CI: 32.0-80.6) respectively. PR patients proceeding to ASCT had a median PFS of 17.2m (95% CI: 14.2-20.2) and 23.1m (95% CI: 16.8-29.4) for those who were MRD- (N=20) and MRD+ (N=7) at D100 respectively, 2-year PFS 17.4% (95% CI: 0.0-33.3) and 17.1% (95% CI: 0.0-33.3) respectively.

Summary/Conclusions: This is the first study to report outcomes of patients stratified to ASCT by depth of response. The overall PFS for the study is shorter than other published trials, most likely due to the inferior outcome for MRD+ patients to induction more adverse FISH lesions (t(4;14), t(14;16), t(14;20), del(17p13), +1q21). The median PFS for ≥VGPR patients who are MRD- and stopped therapy was similar to that in PR patients achieving MRD- status post-ASCT. The PFS for ASCT was relatively short, reflecting the need for maintenance post-ASCT. The median PFS for ≥VGPR patients who are MRD- and stopped therapy was similar to that in PR patients achieving MRD- status post-ASCT. The PFS for ASCT was relatively short, reflecting the need for maintenance post-ASCT.
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Background: Lytic lesions occur in the majority of patients with multiple myeloma (MM) and represent one of the criteria for starting therapy. In the past, whole body X-ray (WBX) represented the method of choice for detecting skeletal abnormalities; today, magnetic resonance imaging (MRI), positron emission tomography (PET) and computed tomography (CT) have been adopted for their higher power in detecting extra-medullary localizations and their higher sensitivity. Nevertheless, which technique would be really the best one is still matter of debate.

Aims: Our single-center retrospective study was designed to compare PET-CT with other imaging techniques (WBX, vertebro column CT and MRI) at the diagnosis and during the follow-up of MM patients. Finally, we assessed a possible predictive/prognostic role of the PET-CT in terms of quality of response and survival.

Methods: We enrolled 160 patients with diagnosed symptomatic (N=149) or smoldering multiple myeloma (N=11) observed at the AOUPI, Pisa, Italy, between January 1996 and December 2015. Eighty-three were male and 77 female; the median age was 70 years (range, 28-85), and half of them presented with low ISS risk score. Forty-five subjects were not eligible to high-dose therapy; 64% of them received bortezomib- and 23% melphalan-based regimens. Patients eligible to high-dose therapy received VAD, TAD or VTD and then one (88%) or two (12%) autologous transplants. At the relapse, lenalidomide (57%) orCarthy (33%) were administered.

Results: Overall, we compared 160 PET-CT, 233 WBX, 106 CT, and 85 MRI exams. At diagnosis, PET-CT allowed detecting skeletal involvement in 18% of cases negative by WBX, in 37% of those CT-negative, and in 10% of those MRI-negative. Sensitivity of PET-CT was superimposable to that of MRI (90%), and higher than that of WBX (60%) and CT (73%). Nevertheless, the specificity was lower for PET-CT and MRI (40%) in respect of CT (51%) and WBX (71%). Analogously to that observed at diagnosis, PET-CT during follow-up showed distinct advantages in terms of sensitivity compared to X-rays (83% vs 60%, respectively). In contrast, PET-CT sensitivity was comparable to that of CT and MRI. As at diagnosis, the specificity was higher for WBX (70%) than for CT, RM and PET-CT (40% for all of these). When PET-CT was correlated to the quality of response, it was significant only in the not transplanted cohort (>PR rate in PET-negative cases 27% vs 32% in the PET-positive group; p=0.016). Nevertheless, PET-CT positivity either at diagnosis or during follow-up did not impact on long-term OS and PFS.

Summary/Conclusions: Our study showed that PET-CT and MRI would represent the techniques of choice in the assessment of bone involvement in MM patients in view of their high and comparable sensitivity. Moreover, PET-CT gives the possibility of a “whole body” analysis in exchange for higher “biologic” cost.

P672 INITIAL PHASE 2 RESULTS OF IBRUTINIB COMBINED WITH BORTEZOMIB/DEXAMETHASONE IN PREVIOUSLY TREATED PATIENTS WITH MULTIPLE MYELOMA

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Background: Bruton’s tyrosine kinase (BTK) is overexpressed in, and has been implicated in the growth and survival of multiple myeloma (MM) cells, providing a rationale for evaluating BTK inhibitors in MM (Yang Cancer Res 2015; Tai Blood 2012). Yang 2015 demonstrated that BTK overexpression (OEs) contributes to blunted responses in MM cells when treated with widely used MM drugs (ie, bortezomib [BTZ], etoposide and doxorubicin). Increased activity of the ABC transporter efflux pump and expression of the ABCB1 transporter protein was seen in BTX OE cells, and subsequent inhibition led to a restoration of BTZ sensitivity in these cells. Ibrutinib (ibr), a first-in-class, once-daily oral inhibitor of BTK, is approved in the US and EU for the treatment of various B-cell malignancies. In the EU, regimens containing BTZ and corticosteroids are a standard of care in MM treatment. A previous study of ibr-bortezomib/dexamethasone (ibr+BTZ+dex) was conducted, an OBSR of 58% in relapsed/refractory MM patients (pts) with a median of 3 prior therapies and 88% refractory to their most recent therapy (Chari ASH 2015), warranting further investigation of ibr in previously treated MM pts.

Aims: To evaluate safety and efficacy of combination ibr+BTZ+dex in previously treated MM pts.

Methods: In this phase 2, open-label, multicenter, European study (PCYC-1139), eligible pts received 1-3 prior therapies and demonstrated disease progression on or following the most recent therapy. Prior BTZ use was permitted provided pts were sensitive (ie, no progression ≤60 days after having achieved minimal response or better). All pts provided informed consent. For cycles 1-8 (21-day cycles), pts received ibr 840mg once daily with BTZ 1.3mg/m2 subcutaneously twice weekly (Days 1, 4, 8, 11) and dex 20mg on day of and after BTZ. For cycles 9-12 (42-day cycles), BTZ was dosed weekly (Days 1, 8, 22, 29). The primary endpoint was PFS with secondary endpoints including safety, ORR, PFS at landmark points, duration of response, and time to progression (TTP).

Results: As of November 21, 2016, 20 pts were enrolled (Table). Median age was 68.5 years (range, 49-96). Median number of prior therapies was 1, with 50% refractory to the most recent therapy and 70% previously exposed to BTZ. Gene expression profiling (GEP) in initial pts indicated high-risk GEP in 35% of pts. Virtual fluorescent in situ hybridization identified 40% of pts with high cytogenetics. Median treatment duration was 2.1 months (range, 0.5-3.7). All pts experienced at least one treatment-emergent adverse event (AE) of any grade. The most common all-grade nonhematologic AEs occurring in >15% (>3 pts) were diarrhea (25%), nausea and vomiting (15%), and hypotension. AEs occurring in ≥10% (>1 pt) were thrombocytopenia (25%), asthenia and pneumonia (15% each), and hyponatremia, abnormal hepatic function, infection, and bone pain (10% each). Three deaths were reported (sudden death in a pt with cardiac history, pneumonia, and myocardial infarction). With early follow-up, 19 pts are evaluable for response with an ORR of 47%, including MR or better in 68%. Updated data will be presented.

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P673 PROGNOSTIC SIGNIFICANCE OF CLONAL CIRCULATING PLASMA CELLS BY MULTI-PARAMETRIC FLOW CYTOMETRY IN PATIENTS WITH LIGHT CHAIN AMYLOIDOSIS UNDERGOING AUTOLOGOUS STEM CELLS TRANSPLANTATION

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Background: Presence of circulating plasma cells (cPCs) prior to autologous stem cell transplant (ASCT) is an adverse prognostic factor in patients with light chain amyloidosis (AL). The predictive value of cPCs prior to ASCT and categorized them as follows: a) Group 1: patients proceeding directly to ASCT without induction therapy and b) Group 2: patients who received induction therapy before ASCT.
Results: There were 78 patients in Group 1 and 52 patients in Group 2. Patients in Group 2 had higher baseline eGFR (60 ml/min), bone marrow plasma cells (BMPC), Mayo stage and were more likely to have active MM compared to patients in Group 1. Table 1 lists baseline characteristics of the patients in Groups 1 and 2. Patients in Group 1 had higher rate of renal involvement. cPCs were detectable in 22% (n=28) of patients at the time of ASCT. More patients in Group 1 had detectable cPCs than in Group 2 (31% vs 8%; p=0.002), likely due to clearance of cPCs with treatment. Data on cPCs at diagnosis in the induction group was available in 14 patients, of whom 57% (n=8) had detectable cPCs vs 31% in the direct ASCT group (p=0.06). 6 of the 8 (75%) patients cleared cPCs with induction therapy. There were no significant differences in patients who had detectable vs undetectable cPCs before transplant, including organ involvement, baseline eGFR, BMPC, and Mayo Stage (data not shown).

In Group 2, both progression free survival (PFS) (10.5 months vs 58 months, p <0.0001) and overall survival (OS) (16 months vs not reached, p <0.0001) were worse in patients who had detectable cPCs compared to those without cPCs (Figure 1). This difference was not seen in Group 1 (OS: not reached vs 98 months, p=0.96; PFS 43 vs 52 months, p=0.74). In multivariate analysis, adjusting for Mayo Stage and induction chemotherapy, there was a trend towards worse OS in patients with detectable cPCs (p=0.06).

Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>cPCs</th>
<th>Median eGFR (mL/min)</th>
<th>Median Age (years)</th>
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Figure 1.

Patients receiving induction chemotherapy before ASCT

Summary/Conclusions: cPCs are cleared after induction treatment in majority of AL patients. Patients who have detectable cPCs prior to proceeding to ASCT after induction have worse PFS and OS than patients without cPCs. On the other hand, presence of cPCs was found not be an adverse prognostic factor in patients proceeding directly to ASCT. This may be due otherwise excellent prognosis in this group, with absence of other high-risk features that are seen in patients who require induction. A limitation of our study is lack of data on cPCs at diagnosis in all patients who received induction therapy.

P674

RENAI IMPAIRMENT IN MYELOMA - PATIENT CHARACTERISTICS, TREATMENT MODALITIES, STEM CELL TRANSPLANT & OUTCOMES FROM THE AUSTRALIAN AND NEW ZEALAND MYELOMA REGISTRY P.J. Ho1,*, E. Moore2, Z. McQuilten2, K. Bergin2, B. Augustson3, H. Blacklock4, TREATMENT MODALITIES, STEM CELL TRANSPLANT & OUTCOMES RENAL IMPAIRMENT IN MYELOMA - PATIENT CHARACTERISTICS, P674

Aims: To assess (1) characteristics of patients with RI at diagnosis - severity of RI, age, risk factors, high-risk features, stage, disease manifestations & performance status, and (2) treatment including induction therapy & autologous stem cell transplant (ASCT) and outcomes.

Methods: Data from newly diagnosed MM patients enrolled in the Australian and New Zealand Myeloma Registry from 1 Feb 2013 to 31 Dec 2016 were analysed.

Results: Of 867 patients, 775 had eGFR available at diagnosis: 34% (287/775) had eGFR <60m/min (22% at 30-60m/min; 6% at 15-30m/min; 6% at <15 m/min). Mean age of patients with RI (<60 m/min) was 72 vs 64 yrs without RI. Diabetes mellitus (DM), a major cause of chronic kidney disease (CKD), was more prevalent in patients with RI: 17% of patients with eGFR <30 m/min compared with 8% >30m/min. Patients with RI (<30m/min) and DM had a similar response to first-line therapy compared to RI without DM (≥PR, 75% vs 82%, p=0.56), with no difference in OS (26 vs 37 mths, p=0.68) or PFS (24 mths, p=0.82). High risk features of FISH (del17p, t(14;16), t(14;16), amp1q21, del13q) & high LDH were more prevalent in RI (6 vs 46, p=0.01). Anemia was more prevalent in RI (44 vs 14%, p<0.001), but bone lesions were less prevalent (52% vs 65%, p=0.001). There was no difference in ECOG performance status. Most patients (87%) received Bortezomib-based therapy in first line (81% RI vs 91% no RI, p<0.001), with no difference with or without ASCT. Response rates (≥PR) were the same in patients with eGFR <60m/min compared with normal renal function (84% vs 85%, p=0.87). PFS & OS decreased with reduction in eGFR (Fig 1). However, patients with eGFR<15m/min had better OS & PFS compared with eGFR 15-30m/min; dialysis in eGFR <15m/min may be a factor. Using age 70 yrs as a common age limit for ASCT, we analysed the effect of ASCT in patients <70 yrs with & without RI. While a smaller proportion of RI patients received ASCT (21% vs 79%, p=0.07), it was performed at all levels of renal function including eGFR <15m/min. In patients with eGFR <60m/min, those who received ASCT had a longer PFS (HR 0.37, 95%CI 0.16-0.88, p=0.03) & OS (HR 0.28, 95%CI 0.08-1.01, p=0.05) compared with no ASCT. The improvement was also seen in severe RI (<30m/min), with a longer PFS (HR 0.21, 95%CI 0.05-0.86, p=0.03) & OS (HR 0.10, 95%CI 0.01-0.82, p=0.03) with ASCT.

Figure 1.

Summary/Conclusions: RI occurred in one-third of newly diagnosed MM. A DM, an underlying risk factor for CKD, was more common in RI patients, but not associated with a difference in outcome. Advanced stage & high risk features were more prevalent in RI patients, but bone disease was less common. RI patients had a shorter PFS and OS, overall correlating with eGFR. However patients with eGFR <15m/min had a better OS than 15-30 m/min, for which dialysis may be a factor. In transplant-eligible patients assessed by age <70 yrs, ASCT was performed in 21% of RI patients, at all levels of renal function. Patients with RI who underwent ASCT had a superior PFS and OS than those who did not have ASCT, including those with severe RI (eGFR <30m/min), supporting the benefit of ASCT in MM patients with RI.

P675


Background: Venetoclax (VEN), an orally available selective small-molecule
BCL-2 inhibitor, induces cell death in multiple myeloma (MM) cells, particularly those with the t(11;14) translocation.

Aims: The objectives of the study are to evaluate safety, PK, recommended phase two dose, and preliminary efficacy of VEN monotherapy in relapsed/refractory (R/R) MM.

Methods: Patients (pts) with relapsed/refractory (R/R) MM received VEN monotherapy in this phase 1 study. Daily VEN was given at 300–1200mg in dose escalation cohorts and 1200mg in the safety expansion. Pts with disease progression (PD) on VEN monotherapy could receive VEN plus dexamethasone and remain on study.

Results: As of 19Aug2016, 66 pts were enrolled. Median age was 63 years and 30 (46%) pts had t(11;14). Median number of prior therapies at start was 13 (range: 1–15); 46 (70%) pts were refractory to bortezomib, 20 (30%) to carfilzomib, 57 (77%) to lenalidomide, 35 (53%) to pomalidomide, and 52 (79%) were refractory to the last prior therapy. Median time on VEN monotherapy was 2.5 months (range: 0.2–23); 17 pts received VEN plus dexamethasone after PD for a median of 13.5 cycles (range: 1–35). Fifty-five (83%) pts discontinued, with 41 due to PD. Common adverse events (AEs) were nausea (47%), diarrhea (36%), vomiting (21%) and grade 3/4 hematologic toxicities [thrombocytopenia (32%), neutropenia (27%), anemia (23%), leukopenia (23%)]. Common serious AEs were pneumonia (8%), sepsis (5%), cough, hypotension, pain, and pyrexia (3% each). There were no events of TLS. Six deaths were reported due to PD, and 1 each due to lung disorder and brain hemorrhage following trauma. Overall response rate (ORR) for all pts on VEN monotherapy was 21% (14/66); 10 (15%) achieved very good partial response (VGPR) or better [2 stringent complete response (sCR); 3 CR; 5 VGP]. For all pts, median time to progression (TTP) was 2.6 months (range: 0.1–15) and duration of response (DoR) was 9.7 months (range: 0.2–23).

A clear difference in responses was seen among pts with t(11;14) vs without [ORR, 40% vs 6%; vVGPR, 27% vs 6%]. For pts with t(11;14), median TTP was 6.6 months [vs 1.9 months for pts without t(11;14)] and median DoR was 9.7 months.

Methods: To determine the tolerability and efficacy of daratumumab in combination with KRd in elderly patients with relapsed/refractory multiple myeloma.

Aims: To explore the tolerability and efficacy of daratumumab in combination with KRd in elderly patients with relapsed/refractory multiple myeloma.

Background: Multiple myeloma (MM) affects mostly elderly people with a median age of 69 years at diagnosis, with 35–40% of patients older than 75. Overall survival (OS) is variable: of patients aged 66–70, 9% survive less than 3 months and 23% survive longer than 10 years. Recently the revised ISS (rISS) has been proposed as a prognostic marker that incorporates ISS, FISH and LDH. Another marker, the SKY92 prognostic gene classifier, was developed in younger, transplant eligible multiple myeloma (MM) patients who were included in the HOVON-65/GMMG-HD4 trial. The SKY92 classifier was thoroughly validated in eight independent cohorts, at the time of its initial publication, and since.

Methods: This is a study of older age (≥75 years) patients with relapsed or refractory multiple myeloma (MM) who are candidates for autologous stem cell transplantation (ASCT) and who are refractory to the last therapy with an ORR <10%, refractory to Daratumumab plus KRd as a first-line treatment, ORR ≥10% for pts without t(11;14) and ≥15% for pts with t(11;14) who were refractory to both the last therapy and lenalidomide, and survival >6 months.

Results: As of 19Aug2016, 178 patients in the analysis for which enough bone marrow was available to perform GEP, had a median age of 73 years. At the time of data cutoff, 25 of 178 patients were elderly patients (≥75 years old). The median OS for the 25 patients classified as SKY92 high-risk was shorter than the median OS of standard-risk patients: SKY92 high-risk 21 months versus SKY92 standard-risk 53 months (hazard ratio (HR)=3.55, 95% confidence interval (CI)=1.77-7.39, p<0.01; Figure 1). The 1-year proportion of patients with high-risk t(11;14) was 6% versus 79% for standard-risk t(11;14) proportion. These findings are comparable to the 10% identified in the initial report of the rISS. Interestingly, the proportion of SKY92 high-risk patients is larger (14%), whereas the median OS associated with these patients is shorter (21 vs 25 months). The SKY92 classifier performed better compared to the rISS as high-risk marker for OS. The 2-year OS rate using the SKY92 classifier was 48% in patients versus 13% in rISS-III (p<0.01). The 2-year progression free survival (PFS) rate was similar for SKY92 high-risk and rISS-III (16% and 17%, respectively). In the multivariate analysis, SKY92, rISS and deletion of 13q were independently associated with OS.
M. Björkholm5, O. Landgren4, S.Y. Kristinsson2

21st Congress of the European Hematology Association

Summary/Conclusions: Here, we compared the SKY92 classifier with revised ISS staging and FISH. These data validate the SKY92 classifier as a robust marker to identify high-risk patients in non-transplant eligible MM patients. In these IMiD treated patients, the SKY92, the revised ISS, and FISH markers such as deletion of 13q retain independent prognostic value.

P678

MULTIPLE MYELOMA AND COMORBIDITY: A POPULATION-BASED STUDY

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21st Congress of the European Hematology Association

Background: The number of multiple myeloma patients has grown with aging populations, and with increasing age the number of comorbidities increases as well. Clinically, it is well known that comorbidity in multiple myeloma patients decreases performance status, increases risk of therapy-related complications and may lead to life-threatening conditions. Currently, the literature on comorbidity in multiple myeloma is very limited and based on small case series. Clinical trials rarely include elderly, frail patients due to eligibility criteria. Population-based studies provide valuable information on survival outcomes in relation to presence/absence of comorbidities in newly diagnosed real-life multiple myeloma patients in the general population.

Aims: To evaluate the prevalence of comorbidities and to study the impact of comorbidities on survival among patients with newly diagnosed multiple myeloma.

Methods: All newly diagnosed patients with multiple myeloma from January 1st 1980 to December 31st 2013 in Sweden were included in the study. Using the Swedish Patient Registry, all discharge diagnosis and discharge listings were gathered from each patient from January 1st 1985. Comorbidity conditions were defined as chronic illnesses which demand life-long treatment or follow-up. Only those diagnoses made prior to multiple myeloma were used. Using ICD 8, 9 and 10 codes, comorbid diseases were identified. Kaplan-Meier curves were used to estimate survival. Risk of death was compared among multiple myeloma patients with a comorbid condition to those without a comorbidity, using Cox’s proportional hazards regression (adjusting for age, gender, year of diagnosis, and other comorbid conditions).

Results: A total of 13,718 patients with multiple myeloma were included in the study and 21 groups of comorbidities were identified. The most common diseases were cancer, hypertension, heart failure, ischemic heart disease and atrial fibrillation. Among all patients, 55% had no prior history of comorbidity, 23% had one comorbidity, 12% had two comorbidities, and 10% had three or more comorbid conditions. Survival was negatively influenced by the number of comorbidities (Figure 1). The risk of death was significantly increased in patients with atrial fibrillation (HR=1.08; 95% CI 1.00-1.16), heart failure (HR=1.50; 95% CI 1.40-1.61), stroke (HR=1.20; 95% CI 1.11-1.30), psychological disease (HR=1.27; 95% CI 1.16-1.39), chronic lung disease (HR=1.22; 95% CI 1.12-1.32), diabetes (HR=1.14; 95% CI 1.04-1.36), peripheral vascular disease (HR=1.26; 95% CI 1.12-1.42), cancer (HR=1.10; 95% CI 1.04-1.16), dementia (HR=1.65; 95% CI 1.38-1.99), paralyisis (HR=1.44; 95% CI 1.15-1.80), inflammatory bowel disease (HR=1.38; 95% CI 1.08-1.74), end stage renal disease (HR=1.57; 95% CI 1.03-2.04), and cirrhosis (HR=1.64; 95% CI 1.10-2.43).

Results:
- 13,718 patients with multiple myeloma were included.
- 21 groups of comorbidities were identified.
- The most common diseases were cancer, hypertension, heart failure, ischemic heart disease, and atrial fibrillation.
- Survival was negatively influenced by the number of comorbidities.
- The risk of death was significantly increased for certain conditions, including atrial fibrillation, heart failure, stroke, psychological disease, chronic lung disease, diabetes, peripheral vascular disease, cancer, dementia, paralyisis, inflammatory bowel disease, end stage renal disease, and cirrhosis.

Table 1. Multivariate survival analysis in the HO87/NM18 trial.

<table>
<thead>
<tr>
<th>Comorbidity</th>
<th>HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td>1.10 (1.04-1.16)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.14 (1.08-1.20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart failure</td>
<td>1.20 (1.11-1.30)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>1.27 (1.16-1.39)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>1.08 (1.00-1.16)</td>
<td>0.041</td>
</tr>
<tr>
<td>Heart failure</td>
<td>1.50 (1.40-1.61)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stroke</td>
<td>1.20 (1.11-1.30)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Psychological disease</td>
<td>1.27 (1.16-1.39)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chronic lung disease</td>
<td>1.22 (1.12-1.32)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.14 (1.04-1.36)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>1.26 (1.12-1.42)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cancer</td>
<td>1.10 (1.04-1.16)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dementia</td>
<td>1.65 (1.38-1.99)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Paralyisis</td>
<td>1.44 (1.15-1.80)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IBD</td>
<td>1.38 (1.08-1.74)</td>
<td>0.008</td>
</tr>
<tr>
<td>End stage renal disease</td>
<td>1.57 (1.03-2.04)</td>
<td>0.035</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>1.64 (1.14-2.35)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Figure 1.

Summary/Conclusions: In this large, population-based study including almost 14,000 patients, we have shown that comorbidities are common among newly diagnosed multiple myeloma patients and that comorbidities are associated with an inferior survival. Importantly, the number of comorbidities showed a dose-response relationship with inferior overall survival. For example, the median overall survival for patients with 3 or more comorbidities was reduced by more than 50% compared to patients without comorbidities. The importance of comorbidities should be taken into account when evaluating patients and deciding on treatment strategies for individuals with multiple myeloma.
The text contains a mix of medical research and clinical trial results. It discusses the use of novel therapies in multiple myeloma, a blood cancer. The text is rich in scientific language and references to clinical studies and methodologies. It mentions the use of next-generation sequencing (NGS) to analyze the clonal heterogeneity of the disease, which is a key aspect in understanding the progression of multiple myeloma.

The text also highlights the importance of therapeutic responses and the role of anti-CD38 antibodies in enhancing the efficacy of existing therapies. It concludes with the need for ongoing research to improve the outcomes for patients with multiple myeloma.
BCL2 EXPRESSION IS A POTENTIAL PREDICTIVE BIOMARKER OF RESPONSE TO VENETOCLAX IN COMBINATION WITH BORTEZOMIB AND DEXAMETHASONE IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background: The anti-apoptotic proteins BCL-2 and MCL-1 have been shown to inhibit apoptosis in myeloma. Venetoclax (VEN) in combination with bortezomib (BTZ) has achieved promising results in relapsed/refractory multiple myeloma (MM). However, clinical responses are variable, likely due to an intrinsically heterogeneous MM population, and only 51% of myeloma cells were eliminated without panobinostat treatment. As of 19 Aug 2016, 66 patients were enrolled on study. Baseline bone marrow aspirate samples were available from 52 patients, of which 45 were evaluable for BCL-2 family gene expression by droplet digital PCR in CD138-selected tumor cells. Correlation between BCL2 (BCL-2), BCL2L1 (BCL-XL) and MCL1 (MCL-1) mRNA expression (log2-transformed copies/ul normalized to housekeeping gene) and preliminary efficacy (overall response rate (ORR), time to disease progression (TTP) and duration of response (DoR)) were examined by Log-rank and Wilcoxon tests for binary biomarkers, and by risk ratio from Cox proportional hazard model for continuous biomarkers.

Results: The ORR was 68% (44/65) for all evaluable patients and 89% (31/35) in patients who had 1-3 prior therapies (31/35). A broad range of BCL2, BCL2L1 and MCL1 expression was observed, however higher BCL2 levels were detected in patients who achieved a partial response (PR) or better (median: 3.01 vs 0.87, p<0.01). Additionally, higher BCL2 levels were observed in patients who had 1-3 previous therapies compared to 4 or more lines of therapy (median: 3.03 vs 0.94, p<0.01). In contrast, no association was observed between BCL2L1 or MCL1 gene expression and response or number of prior therapies. Bootstrapping and aggregating thresholds from trees was used to estimate a threshold value for BCL2 expression that would provide optimum selection of patients to have a response. On a small, seventeen of 18 patients with high BCL2 expression (>3.0) achieved at least a PR (ORR 94%), with 12 patients (66%) achieving VGPR or better (Figure 1). Sixteen of 27 patients with low BCL2 expression achieved at least a PR (ORR 59%), with 6 patients (22%) achieving a VGPR or better. Median TTP (11.6 vs 5.7 months) and DoR (10.2 vs 4.9 months) were also higher in patients with high BCL2 expression. Responses in high BCL2 expressing patients were independent of cytogenetic status as determined by interphase FISH analysis, including t(11;14), t(4;14), del(13q) and del(17p).

Summary/Conclusions: Targeting BCL-2 and MCL-1 with the combination of VEN, BTZ and dexamethasone provides a unique approach for MM treatment. Efficacy results in tumors expressing high BCL2 levels, including 94% ORR, provide supportive evidence for the evaluation of this combination regimen in the ongoing phase 3 study (NCT02755597) in R/R MM.

P683

THE IMPACT OF THE INTRODUCTION OF BORTEZOMIB ON DIALYSIS INDEPENDENCE IN MULTIPLE MYELOMA PATIENTS WITH RENAL FAILURE: A NATIONWIDE DUTCH POPULATION-BASED STUDY

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Background: Renal insufficiency is common at presentation in patients with multiple myeloma (MM) and associated with a poor survival. Approximately 10% of the patients require dialysis. Studies have shown that the novel agent bortezomib has a positive effect on recovery of renal function in MM patients with renal insufficiency.

Aims: The aim of this study is to determine the effect of the revised guideline, including the introduction of bortezomib as first line treatment in MM patients with dialysis dependence, on renal function recovery.

Methods: All patients on renal replacement therapy (RRT) in the Netherlands are registered in the Dutch registry Renine. Data on age, gender, start date of RRT, time and switch of RRT, primary renal diagnosis, date of death and cause of death are collected. In this nationwide population-based study, we selected all patients with MM registered in Renine between January 2002 and January 2016. No information regarding therapy of MM is provided in Renine. In March 2010, bortezomib was advised as first-line treatment in patients suffering from MM with renal impairment in the Dutch guidelines. Therefore, we divided our cohort in two periods: before the bortezomib guideline (January 1, 2002 till March 29, 2010) and after introduction of the bortezomib guideline (March 29, 2010 till January 1, 2016). Kaplan-Meier and Cox proportional hazards modelling were used to identify significant indicators for dialysis independency.

Results: A total of 700 patients were included in the study (422 patients pre-bortezomib and 278 after bortezomib introduction). In the period after the introduction of bortezomib 15% of patients became dialysis independent compared to 8% in the pre-bortezomib period (HRadj.=2.1 (95% CI 1.0–4.2) and HRadj.=5.7 (95% CI 2.5–13.2), respectively).

Summary/Conclusions: In this nationwide population-based study, covering all patients with MM and concomitant renal failure, almost a two-fold increase of patients becoming dialysis independent occurred in the period after the introduction of bortezomib compared to the pre-bortezomib period. This was even more prominent when age was < 75 years and LCDD was the primary renal disease.
TTK and faint light chain deposition but MS confirmed the diagnosis of TTR amyloidosis (TTR gene non mutated). The two remaining patients were diagnosed of AL amyloidosis by IHC unequivocally in other tissues. Mean value of NT-proBNP (N-terminal-natriuretic peptide) in patients with AL amyloidosis was 7730 pg/ml for those with a positive uptake in the scintigraphy and 9990 pg/ml for patients with negative uptake. Summary/Conclusions: Cardiac 99mTc-DPD SC has been described as a useful technique in the differential diagnosis between AL and TTR amyloidosis. However, up to 30% of cases of AL amyloidosis show some degree of uptake and 10% show a pattern consistent with TTR amyloidosis (biventricular uptake and PS 2-3). We believe that the combination with conventional methods is mandatory in cases of cardiac amyloidosis with abnormal FLC ratio and positive biventricular 99mTc-DPD uptake. We did not find any correlation between positive uptake in the SC and NT-proBNP values, as it has been recently suggested.

P685
MYOCARDIAL UPTAKE OF 99mTc-DPD IN PATIENTS WITH AL AMYLOIDOSIS
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Background: Cardiac 99mTc-DPD scintigraphy has been described as a myocardial uptake in SC. MS is not routinely available in most centers and results may be ambiguous. We consider that MS is mandatory in cases of cardiac amyloidosis with abnormal FLC ratio and positive biventricular 99mTc-DPD uptake. We did not find any correlation between positive uptake in the SC and NT-proBNP values, as it has been recently suggested.

Aims: The aim of this study was to report the efficacy of pomalidomide and dexamethasone in patients with multiple myeloma-associated AL amyloidosis.

Methods: We performed 704 SC for different indications during the study period. Adverse events were observed in 5 (17%) of subjects: skin rash and confusion in one patient each and mild increase in serum creatinine in 3 (10%, resolved with the decrease of the dose of pomalidomide). The median number of prior treatments was 3 (2-4). All patients previously received lenalidomide and an alkylating agent, only 3 patients were not exposed to bortezomib, due to severe peripheral nervous system involvement, 10 (33%) underwent autologous stem cell transplant and 9 (30%) received previous thalidomide and an alkylating agent, only 3 patients were not exposed to bortezomib, due to severe peripheral nervous system involvement, 10 (33%) underwent autologous stem cell transplant and 9 (30%) received previous thalidomide-based regimens. The median number of PDX cycles performed was 4 (range: 1-11). Median follow-up of living patients was 5 months (IQR: 3.5-11.5 months) and 13 (43%) patients died due to progressive disease. Fourteen patients (47%) achieved at least partial response, with 1 complete remission (CR), and very good partial responses (VGPR) in 2 cases (6%). Cardiac amyloidosis was observed in 1 of 5 patients with measurable NT-proBNP (20%), but this can be underestimated due to the pomalidomide-related increase of NT-proBNP, and renal response in 3 of the 11 evaluable patients (27%).

Summary/Conclusions: The combination of pomalidomide and dexamethasone is well tolerated and effective in multiple myeloma-associated AL amyloidosis and can be a valuable rescue option in this high-risk population.
Background: To contextualize the benefit of novel agents such as daratumumab (DARA) monotherapy for the treatment of patients with heavily pre-treated and highly refractory multiple myeloma (MM), it is critical to understand the real-world outcomes of this patient population on current standard of care (SOC) therapies. To determine the comparative effectiveness of DARA vs real-world SOC, an adjusted comparison was conducted utilizing data from the DARA monotherapy trials and the International Myeloma Foundation (IMF) chart review.

Aims: The objective of this analysis is to update the adjusted comparison to include new Swedish patients from the IMF chart review.

Methods: Data for patients treated with DARA 16mg/kg monotherapy were available from clinical trials MMY2002 (n=106) and GENSO1 (n=42), while patients treated with SOC therapies were derived from the IMF chart review of patients with MM who had ≥3 prior lines of therapy and were double refractory to a minimum of 1 immunomodulatory drug (IMiD) (n=550, original 510, additional Swedish patients 40). Patients from the IMF cohort who moved into further treatment lines after the line therapy where they fulfilled inclusion criteria, contributed information to the analysis for multiple lines of therapy, with baseline defined as the date of initiation of the actual treatment line, resulting in a minimum of 963 treatment lines from 550 patients. The relative treatment effect of DARA versus SOC was estimated using multivariate Cox regression analyses. The methodology utilized individual patient data to compare overall survival (OS). The covariates included were age, gender, prior lines of therapy, albumin, beta-2 microglobulin, prior exposure to pomalidomide and carfilzomib, and double refractory status. Clustering of observations at the treatment-line level within patients was controlled for using the robust sandwich estimate for the covariance matrix. Statistical significance testing was performed using a two-tailed P-value <0.05, and all comparisons between treatment groups were reported with hazard ratios (HRs) and 95% confidence intervals (CIs).

Results: After adjustment for differences in baseline characteristics included in the multivariate model between the DARA and SOC groups, results showed a significant improvement in favor of DARA compared with SOC for OS (HR=0.42 [95% CI 0.31–0.57]). When limiting the comparative analysis to European patients from the IMF cohort (n=341), results for OS are very similar (HR=0.40 [95% CI 0.28-0.58]).

Summary/Conclusions: Findings from the regression analyses using the updated IMF dataset were consistent with results from the previous analysis and suggest that DARA is associated with significant gains in OS compared with SOC therapies for patients with heavily pre-treated and highly refractory MM. Findings for a European subset from the IMF dataset were similar to results from the entire cohort.

References:

P688

PREDICTORS OF EARLY DEATH RELATED TO ACTIVE MULTIPLE MYELOMA IN ELDERLY PATIENTS RECEIVING OPTIMIZED FRONTLINE TREATMENT COMBINATIONS

P. Rodriguez Otero1, M.V. Mateos 2, M.-L. Joaquin3, H. Miguel Teodoro4, 5.

Background: Multiple Myeloma (MM) is predominantly a disease of the elderly and the outcome of these patients is poorer than that of transplant candidates. It is well established that those considered frail or unfit have a dismal prognosis, however, even within fit patients, such as those included in clinical trials, there is substantial proportion of early deaths (within the first 2 years after diagnosis).

Methods: 497 NDMM not transplant candidates treated in two prospective GEM-PETHMA trials were included in the study: GEM05MAS65 (n=260) used frontline treatment with either bortezomib-melphalan-prednisone (VMP) or bortezomb-thalidomide-prednisone followed by maintenance with bortezomib, thalidomide or bortezomb, prednisone; the GEM2010MAS65 (n=239) compared induction with sequential or alternating cycles of VMP + lenalidomide-dexamethasone. The event was defined as death related to active MM within 2 years from diagnosis, either because of disease progression or early death due to absence of response.

Table 1.

Results: From the 497 patients included, 77 (15%) patients died within 2 years from diagnosis due to active MM. When we compared this latter cohort with the remaining patients, the profile of the high risk group was characterized (Table 1) by a higher proportion of patients >75 years, advanced ISS and R- ISS stage, higher β2-microglobulin (>3.5 and 5.5mg/dl) and abnormal LDH; increased incidence of high-risk cytogenetic features (HR CA), CD45+ clonal plasma cells, and lower incidence of CD27+ MM phenotype.

References:

P689

PREVENTION OF EARLY DEATH RELATED TO ACTIVE MULTIPLE MYELOMA IN ELDERLY PATIENTS RECEIVING OPTIMIZED FRONTLINE TREATMENT COMBINATIONS

P. Rodriguez Otero1, M.V. Mateos 2, M.-L. Joaquin3, H. Miguel Teodoro4, 5.

Background: Multiple Myeloma (MM) is predominantly a disease of the elderly and the outcome of these patients is poorer than that of transplant candidates. It is well established that those considered frail or unfit have a dismal prognosis, however, even within fit patients, such as those included in clinical trials, there is substantial proportion of early deaths (within the first 2 years after diagnosis).

Methods: 497 NDMM not transplant candidates treated in two prospective GEM-PETHMA trials were included in the study: GEM05MAS65 (n=260) used frontline treatment with either bortezomib-melphalan-prednisone (VMP) or bortezomb-thalidomide-prednisone followed by maintenance with bortezomib, thalidomide or bortezomb, prednisone; the GEM2010MAS65 (n=239) compared induction with sequential or alternating cycles of VMP + lenalidomide-dexamethasone. The event was defined as death related to active MM within 2 years from diagnosis, either because of disease progression or early death due to absence of response.

Table 1.

Results: From the 497 patients included, 77 (15%) patients died within 2 years from diagnosis due to active MM. When we compared this latter cohort with the remaining patients, the profile of the high risk group was characterized (Table 1) by a higher proportion of patients >75 years, advanced ISS and R- ISS stage, higher β2-microglobulin (>3.5 and 5.5mg/dl) and abnormal LDH; increased incidence of high-risk cytogenetic features (HR CA), CD45+ clonal plasma cells, and lower incidence of CD27+ MM phenotype.

References:

P689

PREVENTION OF EARLY DEATH RELATED TO ACTIVE MULTIPLE MYELOMA IN ELDERLY PATIENTS RECEIVING OPTIMIZED FRONTLINE TREATMENT COMBINATIONS

P. Rodriguez Otero1, M.V. Mateos 2, M.-L. Joaquin3, H. Miguel Teodoro4, 5.

Background: Multiple Myeloma (MM) is predominantly a disease of the elderly and the outcome of these patients is poorer than that of transplant candidates. It is well established that those considered frail or unfit have a dismal prognosis, however, even within fit patients, such as those included in clinical trials, there is substantial proportion of early deaths (within the first 2 years after diagnosis).

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Table 1.
unsR (duration of response (≥PR) <6 months) to the baseline score we were able to build a new score in which the unsR had a 3 points weight. A score punctuation ≥4 segregates a subgroup of patients with poor outcome (PPV: 83.3%, the NPV: 84.02%).

Summary/Conclusions: The risk of early death due to active disease in elderly patients was related to four independent prognostic factors: age >75y, high LDH levels, advanced ISS, and presence of HR CA. A score ≥4 identify a subgroup of patients with high probability of death within 2 years despite optimized treatment.

Myeloproliferative neoplasms - Biology

P689

MPL ACTIVATION DIRECTLY INDUCES FIBROCYTE DIFFERENTIATION TO CAUSE MYELOFIBROSIS

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Background: Myelofibrosis (MF) may be caused by various pathogenic mechanisms, such as elevated circulating cytokine levels, cellular interactions, and genetic mutations. However, the underlying mechanism of MF remains unknown. A recent study showed that the neoplastic clone of fibrocytes, spindle-shaped fibroblast-like blood cells derived from monocyte lineage, was essential in primary MF pathogenesis; serum amyloid P, which suppressed fibrocyte differentiation, markedly improved survival and MF in a murine xenograft model (J Exp Med 2016; 213: 1723-1740). Regarding cytokines, the thrombopoietin (TPO) signaling pathway was assumed to be closely associated with promoting MF. Mice transplanted with TPO-overexpressing bone marrow cells showed symptoms such as MF and splenomegaly (Blood 1997; 90: 4369-4383). Romiplostim (Rom), a TPO-receptor agonist, induced MF in rats and some immune thrombocytopenic purpura patients (Blood 2005; 114: 3749-3756). Fibrocytes and TPO played certain roles in MF pathogenesis, but the nature of their relationship remains unknown.

Aims: We investigated the relationship between myeloproliferative leukemia protein (MPL; TPO receptor) activation and fibrocyte differentiation in promoting MF. The secondary goal was to discover a unique fibrocyte marker in monocyte or macrophage population.

Methods: Murine fibrocyte cell lines were established from transgenic mice harboring the temperature-sensitive large T-antigen gene of simian virus 40 under IL-13 and M-CSF conditions. Murine fibrocyte cell lines and human peripheral blood mononuclear cells (PBMCs) were cultured with or without Rom to evaluate if MPL activation promoted fibrocyte differentiation, and the ratio of spindle-shaped cells was calculated. Rom was administered on day 1 and 8 to induce an MF-like phenotype in C57BL/6J mice, and clodronate liposomes (CLs; day −4, −1, 4, and 7) were used to eliminate monocytes and macrophages.

Results: Flow cytometric analysis revealed that all murine fibrocyte cell lines stained positive for fibrocyte cell markers, including collagen I, CD45, CD34, CD11b, and CD68. Murine fibrocyte cell lines expressed MPL and responded to Rom or murine TPO to differentiate into mature fibrocytes, specifically with a longer spindle shape and stronger collagen I expression than when cultured with IL-13 and M-CSF alone. Rom also increased the number of mice spleen cell fibrocyte colonies in the presence of IL-13 and M-CSF. The administration of 1mg/kg of Rom once a week induced an MF-like phenotype in all mice within 2–3 weeks and increased the number of fibrocytes in the spleen. Treatment with CLs eliminated fibrocyte precursors and prevented severe MF and splenomegaly. Human cultured fibrocytes also expressed MPL, and Rom increased the number of spindle-shaped fibrocytes induced from human PBMCs. The SLAMF7high MPLhigh subpopulation was clearly separated from the SLAMF7low MPLlow population in human CD14+ monocytes. A significantly higher frequency of fibrocyte differentiation was observed in the SLAMF7high MPLhigh population. The number of SLAMF7high MPLhigh cells was significantly greater in MF patients than in healthy donors. Conversely, their numbers did not increase in MF patients treated with ruxolitinib.

Summary/Conclusions: MPL activation directly induced fibrocyte differentiation from monocytes and macrophages expressing MPL, and the elimination of these cells reversed the MF phenotype. Our findings confirmed a link between fibrocytes and the TPO/MPL signaling pathway and indicated that the combination of MPL and SLAMF7 could be a useful fibrocyte marker in monocytes or macrophages.

P690

ENGRAFTMENT OF PRIMARY MYELOFIBROSIS BONE MARROW-DERIVED CD14+ MONOCYTES IN NOD-SCID-γ MICE

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Background: Progressive bone marrow (BM) fibrosis in patients with PMF is thought to arise from non-hematopoietic stromal cells stimulated by overpro-
duced growth factors. However, in other tissues and organs, fibrosis is associated with the occurrence of fibrocytes, which express markers of both hematopoietic and stromal cells. Recently, we have reported that clonal neoplastic fibrocytes play a role in the induction of BM fibrosis in primary myelofibrosis (PfMR) (Verstovsek, J Exp Med. 2016). We demonstrated that the BM of PMF patients harbors more neoplastic, functionally distinct fibrocytes and fewer BM and hematologically normal bone marrow fibrocytes. In addition, we detected an overabundance of fibrocytes in the BM and spleen of an established PMF mouse model and a xenograft mouse model of PMF created using BM-derived low-density cells from patients with PMF. Aims: Fibrocytes, which make up <1% of BM cells, differentiate from a subpopulation of BM monocytes and are recruited to sites of organ damage where they regulate tissue repair. We hypothesized that clonal neoplastic CD14+ monocytes may play a role in the induction of BM fibrosis in PMF. Methods: To test this hypothesis, we transplanted NSG mice (NOD/ScidNOG.Cg-prkdcscidIj2tgIlw1Wjl/SJZ) with sorted CD14+ monocytes from patients with JAK2V617F-positive PMF or donors with hematologically normal BM. Results: Here, we show that BM-derived CD14+ cells from patients with JAK2V617F-positive PMF or donors with hematologically normal BM engrafted in NMSCs. Transplanted NSG mice with PMF BM-derived CD14+ monocytes developed a myelofibrosis-like phenotype with reticulin fibrosis and abundant neoplastic (JAK2V617F) fibrocytes in the BM and spleen. Two months after transplantation, we detected a subpopulation of CD45+ and CD68+ cells within the HLA+ population of BM cells. In addition, we found dysplastic megakaryocytes in the BM and spleen of the CD14+ transplanted mice. Immunohistochemistry staining of paraffin embedded BM sections did not detect CD3, CD19, or CD34 cells. However, staining with anti-human CD42b antibodies detected human megakaryocytes, suggesting that the dysplastic megakaryocytes detected in PMF CD14+ transplanted NSG mice are human-derived. Summary/Conclusions: Taken together, our data suggest that neoplastic CD14+ monocytes contribute to the induction of BM fibrosis in PMF. What role CD14-derived megakaryocytes play in the pathogenesis of PMF remains to be determined.

P692 QUANTITATIVE PROTEOME HETEROGENEITY IN MYELOPROLIFERATIVE NEOPLASM SUBTYPES AND ASSOCIATION WITH JAK2 MUTATION STATUS

Aims: The aims of this study were to evaluate the effects of the switch control inhibitor DCC-2618 on proliferation and survival of neoplastic MC and other kinetic activities of KIT D816V. Methods: We have established an in vitro model system that recapitulates the megakaryocytosis caused by mutant JAK2, which should be useful tool for the examination of therapeutic strategies against MPN patients harboring CALR mutation.

Summary/Conclusions: We have established an in vitro model system that recapitulates the megakaryocytosis caused by mutant CALR, which should be useful tool for the examination of therapeutic strategies against MPN patients harboring CALR mutation.

P693 THE NOVEL SWITCH CONTROL INHIBITOR DCC-2618 COUNTERACTS GROWTH AND SURVIVAL OF VARIOUS NEOPLASTIC CELLS, INCLUDING MAST CELLS, EOSINOPHILS, AND MONOCYTES, IN PATIENTS WITH SYSTEMIC mastocytosis

Aims: The novel switch control inhibitor DCC-2618 was shown to be involved in calcium homeostasis and apoptotic signaling, was overexpressed in JAK2V617F-positive granulocytes compared with JAK2 wild-type and independently of the JAK2V617F allele burden. Results: A number of differentially expressed proteins were identified with the most frequent being members of the RAS GTPase family and oxidative stress response proteins. Subsequent analysis found that calreticulin (CALR), known to be involved in calcium homeostasis and apoptotic signaling, was overexpressed in JAK2V617F-positive granulocytes compared with JAK2 wild-type and independently of the JAK2V617F allele burden. Finally it was demonstrated, in a Ba/F3 cell model, that increased calreticulin expression was directly linked to JAK2 kinase activity and could be regulated by JAK2 kinase inhibitors. Summary/Conclusions: In conclusion, these results reveal proteome alterations in MPN granulocytes depending on the genotype and phenotype of patients, highlighting new oncogenic mechanisms associated with JAK2 mutations and overexpression of calreticulin.
MOLM-13, MV4-11, KG-1 and U-937, the eosinophilic leukemia cell line EOL-1, human cultured umbilical vein endothelial cells (HUVEC), the microvascular human endothelial cell line HMEC-1 and primary neoplastic cells obtained from patients with AML, chronic myelomonocytic leukemia (CMMML) and (clonal or reactive) hypereosinophilia were used. Cell proliferation was quantified by 3H-thymidine uptake. Apoptosis was determined by flow cytometry and light microscopy of phosphorimunostaining of kit and BTK was analyzed by Western blotting. The effects of DCC-2618 on histamine secretion in basophils (BA) were analyzed by histamine release assay.

Results: DCC-2618 was found to block the proliferation of all MC lines tested, with lower IC50 values measured in KIT D816V-negative HMC-1.1 cells (12±3 nM) and ROSAkitWT cells (4±1 nM) than in KIT D816V-positive HMC-1.2 cells (123±36 nM), ROSAkitTDB16V cells (18±6±5 nM), and the multi-resistant MC line MCFP-1. The DCC-2618-metabolite DP-5439 showed comparable growth-inhibitory effects in all cell lines tested. DCC-2618 was also found to inhibit proliferation of primary neoplastic MC obtained from patients with AML, CMML, MGUS or ASAHM and MCL (IC50: 83-460 nM). DCC-2618 induced apoptosis and blocked tyrosine phosphorylation of KIT in all MC lines tested. We were also able to show that DCC-2618 inhibits proliferation and survival in the eosinophilic leukemia cell line EOL-1 (IC50 1.8±1.3 nM) and the FLT3 ITD-mutated AML cell lines MV4-11 (IC50 147±60 nm) and MOLM-13 (IC50 132±55 nM). In addition, DCC-2618 was found to block proliferation in primary leukemic cells in patients with monoblastic AML and CMMML which are the most prevalent types of AHM in advanced SM. DCC-2618 was also found to inhibit growth of cultured human vascular endothelial cells, suggesting that the drug may also counteract SM-related angiogenesis. Finally, DCC-2618 was found to inhibit antibody-induced histamine release from normal BA in a dose-dependent manner (IC50: 1-10 µM).

Summary/Conclusions: DCC-2618 is a new potent switch control TKI that counteracts growth and survival of neoplastic MC, leukaemic monocytes, AML blasts, eosinophils, and endothelial cells in vitro. Whether DCC-2618 is able to block growth of neoplastic MC and other involved lineages in patients with advanced SM is currently being ascertained in a clinical trial (NCTO2571036).

P694

DISTRIBUTION OF MUTATIONS IN DRIVER AND NON-DRIVER GENES ACCORDING TO CLONAL HEMATOPOIESIS IN ESSENTIAL THROMBOCYTHEMIA (ET) AND POLYCYTHEMIA VERA (PV)

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Background: Essential thrombocytemia (ET) and polycythemia vera (PV) are clonal myeloid disorders that originate from a multipotential hematopoietic stem cell. Although most women with PV and ET have mutations in JAK2 V617F, CALR or MPL, the proportion of patients presenting clonal hematopoiesis by X chromosome inactivation patterns (XCIP) is variable and its relationship with the presence of non-driver mutations is not well known.

Aims: To study the distribution and dominance of driver and non-driver mutations in the development of clonal hematopoiesis.

Methods: One hundred and twenty-six women (PV n=33, ET n=93) with an informative result of XCIP based on HUMARA assessment were included in the study. HUMARA analysis was performed by studying the degree of methylation of exon 1 in granulocytes and lymphocytes. Somatic mutations were studied in DNA extracted from granulocytes by NGS using a panel of 51 myeloid-related genes.

Results: Median age of patients at the time of HUMARA analysis was 64 years (range 21-92). Mutations in JAK2 were present in 62% of them, CALR in 11%, MPL in 8% and 14% were triple negative (TN). Non-driver mutations were detected in 65% of patients (17 PV and 28 ET). The most frequently mutated genes were TET2 (16%), DNMT3A (8%), ASXL1 (5%), SF3B1 (5%), EZH2 (2%) and RUNX1 (2%). The mutation with the highest variant allele frequency (VAF) was considered the dominant mutation and it corresponded to a driver mutation in 92 patients (JAK2 n=70, CALR n=13, MPL n=8) and a non-driver mutation in 9 patients (PV n=9, ET n=9). We observed 22 combinations with ISM, ASM or ASM-AHN and MCL (IC50: 83-460 nM). DCC-2618 induced apoptosis and blocked tyrosine phosphorylation of KIT in all MC lines tested. We were also able to show that DCC-2618 inhibits proliferation and survival in the eosinophilic leukemia cell line EOL-1 (IC50 1.8±1.3 nM) and the FLT3 ITD-mutated AML cell lines MV4-11 (IC50 147±60 nm) and MOLM-13 (IC50 132±55 nM). In addition, DCC-2618 was found to block proliferation in primary leukemic cells in patients with monoblastic AML and CMMML which are the most prevalent types of AHM in advanced SM. DCC-2618 was also found to inhibit growth of cultured human vascular endothelial cells, suggesting that the drug may also counteract SM-related angiogenesis. Finally, DCC-2618 was found to inhibit antibody-induced histamine release from normal BA in a dose-dependent manner (IC50: 1-10 µM).

Summary/Conclusions: DCC-2618 is a new potent switch control TKI that counteracts growth and survival of neoplastic MC, leukaemic monocytes, AML blasts, eosinophils, and endothelial cells in vitro. Whether DCC-2618 is able to block growth of neoplastic MC and other involved lineages in patients with advanced SM is currently being ascertained in a clinical trial (NCTO2571036).

P695

RUXOLITINIB/NILOTINIB/PREDNISOLONE COMBINATION: A PROMISING NOVEL TREATMENT FOR MYELOFIBROSIS

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Background: Myelofibrosis (MF) is the myeloproliferative neoplasm chromosome Ph- negative with worst prognosis. MF is characterized by stem cell-derived clonal myeloproliferative and reactive cytokine-driven inflammatory bone marrow fibrosis. Ruxolitinib is the first line treatment for MF. It was associated with significant reduction in symptomatic splenomegaly and improved constitutional symptoms. In a previous work (Arenas et al. Blood Volume 122, Issue 21 (ASH Annual Meeting Abstract)) we identified a set of promising synergistic drugs combinations for a ruxolitinib. Nilotinib and prednisolone were selected from them.

Aims: The aim of this work is the study the effect of the combination of ruxolitinib, nilotinib and prednisolone in hematopoietic progenitor cells from patients with MF.

Methods: A ruxolitinib, nilotinib and prednisolone dose-response curves and synergistic studies were performed in hematopoietic progenitors CD34+ from five MF patients. We studied the molecular effect of single drugs and in combination on SET2 cell line with western blot. To adress the antifibrogenic activity of the drugs and their combinations, we pre-incubated HS72 cultures with 100nM of ruxolitinib, 1 µM of nilotinib, 1 µM of prednisolone or their combination during 1 h. After that, we added 2mg/mL TGF-β during 24h to induce fibrogenesis. Finally, the collagen I expression was evaluated by immunocytochemistry (ICC).

Results: The effects of ruxolitinib, nilotinib and prednisolone resulted in an EC50 value of 55nM, 6.6µM and 13.1µM, respectively. A combination index (CI) of less than 1 indicated synergy. All combination had a synergistic behavior (Table 1); moreover, there were two combinations whose CI from all samples were below 1. The most frequent combination was: 32nM ruxolitinib plus 1.6 µM nilotinib and 0.8 µM prednisolone (CI=0.25±0.11) and 32nM ruxolitinib plus 0.8 µM prednisolone (CI=0.45±0.11). The JAK/STAT signaling pathway was inhibited: the phosphorylation of STAT5 at 30 min and maintained at 3 hours. The MAPK signaling pathway was inhibited: the phosphorylation of p38 and ERK at 30 min and 3 hours. The JAK/STAT signaling pathway was inhibited: the phosphorylation of STAT5 at 30 min and maintained at 3 hours. The MAPK signaling pathway was inhibited: the phosphorylation of p38 and ERK at 30 min and 3 hours. The overall proliferation inhibition was 77 ± 1.64% in the combination of ruxolitinib, nilotinib and prednisolone compared to control.

Figure 1
% (p<value<0.05) by ruxolitinib, 42.6±14.4 % by RN and 70.8±11.2 % by RN (p=value<0.001). The inhibition was maintained at 3 hours by ruxolitinib (71.3±18.9%) (p<value<0.05). The Akt/Pi3K signaling pathway seemed to begin to inhibit at 3 hours by ruxolitinib (57±25.2%), nilotinib (38±26.8%), RN (30.5±24.03%) and RNP (37±16.5%). Then, the anti-fibrogenic activity of the drugs and their combinations were studied. Nilotinib reduced the mRNA expression of HS27 (30.5±24.03%) and RNP (37.4±16.5%) at 3 and 6 hours with combination with ruxolitinib 48±12.9% (p<value<0.05) and prednisolone (RNP: 37.8±1.9%) (p<value<0.05). These results were corroborated by ICC: the inhibition of expression of collagen I was more intense if the HS27 were treated with nilotinib or RN (figure 1).

Summary/Conclusions: In conclusion, ruxolitinib, nilotinib, prednisolone and their combinations had a synergistic behavior to control the proliferation of myelofibrotic MF cells; moreover, they had anti-fibrotic activity in fibroblasts cells. For these reasons, the combined ruxolitinib/nilotinib/prednisolone could be a promising therapy to MF and support an ongoing clinical trial in MF patients.

P696
INTERLABORATORY ASSESSMENT OF MUTATION DETECTION IN MYELOID MALIGNANCIES BY TARGETED NEXT-GENERATION SEQUENCING
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Background: Next-generation sequencing (NGS) technology is being implemented in clinical practice for assessing the mutational status of myeloid neoplasms. The Working Group on Molecular Biology from the Spanish Society of Hematology has performed an interlaboratory assessment of gene mutation analysis by targeted NGS using myeloid panels.

Aims: To assess the technical performance of mutation detection by targeted NGS using myeloid panels.

Methods: The technical comparison was established on two rounds with samples previously analysed using NGS panels, Sanger sequencing and/or fragment analysis. First, four DNA samples (S1-S4) from AML patients were shared among 14 laboratories. The center of origin had previously characterized and confirmed: for the first round, 14 relevant mutations in 10 genes; and for the second round 17 relevant mutations in 7 genes. Each center performed library preparation, sequencing and blind variant analysis following their own routine practice. Detected variants and data regarding main methodological parameters were collected. Detection rate was calculated as the number of laboratories that sequenced the particular gene region. No attempt was made to optimize the performance of the different platforms.

Results: Eight different gene panels were used for library preparation (pre-decided in 10 labs and custom in 4). The main current approach was amplicon enrichment (11/14, 78.6%) and only 3/14 laboratories (21.4%) used capture-based methods. Sequencing was performed with Illumina devices in 9/14 laboratories. The technical comparison was 2353 reads (range 275-17096). Results are summarized in the table. Overall, most variants were detected by Sanger sequencing. The percentage of variants detected was considered as a quality mark for the laboratories.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Gene</th>
<th>CDS</th>
<th>AA</th>
<th>% of NGS</th>
<th>% of Sanger</th>
<th>% of Fragment</th>
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<tr>
<td>S1</td>
<td>CALR</td>
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<td>S2</td>
<td>ASPA</td>
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<td>S4</td>
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Summary/Conclusions: The mutation analysis by targeted NGS in myeloid malignancies is highly reproducible between laboratories and allows a comparison for characterization purposes. However, new sequencing indels, low frequency mutations (<10%), ASXL1 p1164fs detection and variant categorization are critical points that have to be addressed to improve the results. Test system validation is crucial for the implementation of NGS technology.

P697
METHYLATION AGE IN MPN PATIENTS AS A CORRELATE FOR DISEASE STATUS, ALLELE BURDEN AND THERAPEUTIC RESPONSE
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Background: Myeloproliferative Neoplasms (MPNs) result from genetic and epigenetic dysregulation. Epigenetic therapies, such as Vorinostat (SAHA, MK-0663), a histone deacetylase inhibitor, have been tested as a therapeutic strategy in these patients. Examining the epigenetic landscape in MPN may provide new insights into predicting therapeutic response and therefore enhance the clinical utility of these agents. Probably the best described epigenetic mechanism is DNA methylation (DNAm); in which methyl groups are added to DNA in a process controlled by DNA methyltransferase enzymes. The extent of methylation is highly reproducible between laboratories and allows a comparison for characterization purposes. However, methylation can be affected by lifestyle or disease on cellular processes. Therefore ‘methylation age’ (MA) is a promising therapy to MF and support an ongoing clinical trial in MF patients. Examining the epigenetic landscape in MPN may provide new insights into predicting therapeutic response and therefore enhance the clinical utility of these agents. Probably the best described epigenetic mechanism is DNA methylation (DNAm); in which methyl groups are added to DNA in a process controlled by DNA methyltransferase enzymes. The extent of methylation is highly reproducible between laboratories and allows a comparison for characterization purposes. However, methylation can be affected by lifestyle or disease on cellular processes. Therefore ‘methylation age’ (MA) is a promising therapy to MF and support an ongoing clinical trial in MF patients.

Aims: Project the sex and age of the individual using MA

Method: A new model using MA was developed. This model included 10 CpG sites in regulatory regions for each gene. The model was validated using data from 50 MPN patients.

Results: The model was able to predict the sex of patients with an accuracy of 97.8% and the age of patients with an accuracy of 92.0%. The model was also able to predict the response to therapy with an accuracy of 86.0%.

Summary/Conclusions: The model was able to predict the sex and age of the individual using MA with high accuracy. The model was also able to predict the response to therapy with an accuracy of 86.0%.
Methods: MA was calculated following pyrosequencing of bisulfite converted DNA from 40 MPN patients on an investigator initiated non randomised open label phase II multicentre study of Vorinostat (EudraCT #2007-005396-49). Paired samples were analysed at trial entry and after 3 months of therapy to calculate their individual MA scores. Validation of methods used and ageing signature calculation was carried out using cell line and healthy volunteer material.

Results: Samples from 19 Essential Thrombocytopenia (ET) and 22 Polycythemia Vera (PV) patients (23 F/17 M) with a mean age of 62 years (range 29-81) were assessed. JAK2V617F was detected in 77.5% (n=31/40). Complete clinical response (CR) was achieved in 8 patients, partial (PR) in 17, and no response (NR) in 15 patients. MA was on average 8.3 years younger than CA (range -3.4 to +1.1) at time of trial entry and 8.2 years younger (range -36.5 to +33.3) after therapy. This difference between MA and CA was greater in ET patients compared to PV, both at trial entry (-14.0 years vs -3.7) and after therapy (-13.0 years vs -4.3). A statistically significant link between JAK2 allele burden and MA was seen: compared to patients with low or no JAK2 allele burden, patients with high JAK2 (>80% at baseline) had an older MA at trial entry (64.2 years vs 44.5, p=0.0007) and after therapy (64.3 years vs 44.6, p=0.0015). This difference was also seen when PV or ET patients were examined separately. Patients with a high JAK2 allele burden tended to have a MA closer to their CA at trial entry (-0.6 years vs -15.3, p=0.0122) and after 3 months therapy (-0.5 years vs -16.2, p=0.0072). Although the cohort size was small, within the ET group, NR compared to PR was associated with a younger MA after therapy (41.4 years vs 56.3, p=0.0156). Within PV, NR compared to PR was associated with a MA that was older than CA both before (+9.2 years vs -14.2 years, p=0.0346) and after therapy (+7.4 years vs -13.9, p=0.0347).

Suggested Conclusions: A link between MA and JAK2 mutant allele burden in MPN patients, suggesting that allele burden not only has a role in clinical phenotype and disease evolution but in the overall methylation landscape of the mutated cells. However, the role of MA with respect to therapeutic response needs to be clarified with further studies required to show its full impact.

P698 ELUCIDATING THE AGE INDUCED HEMATOPOIETIC CELL-INTRINSIC AND EXTRINSIC MECHANISMS IN MYELOPROLIFERATIVE NEOPLASMS INITIATION AND PROGRESSION
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Background: The number of detectable somatic mutations increase with age, but this increase is surpassed by the rise in the incidence of cancer in older people. The underlying mechanisms for this disparity remain to be elucidated. Myeloproliferative neoplasm (MPN) is an ideal malignancy model disease to study clonal hematopoiesis, disease initiation and progression during natural aging because the majority of the relevant mutations (such as JAK2 V617F) are catalogued, the disease evolves and progresses slowly allowing the collection of serial samples, and an inducible transgenic mouse models for the disease have been established. Nonetheless, the prevalent occurrence of such clonal events in aged individuals brings up the question, which age-associated factors contribute to initiate hematologic malignancies and what are the rate limiting steps attributable for age-induced myeloid malignancies? We hypothesise that age-induced myeloproliferations provide a context that favours a coproduction of new mutations, selection for pre-malignant clones, and that activation of mutant JAK2 further augments these changes for increased MPN incidence in aged individuals. Thus, delineation of age associated cellular and molecular mechanisms attributable for increased prevalence of myeloid malignancies will be essential for the development of strategies for early detection and therapeutic targeting of myeloid malignancies.

Aims: The goal of this proposal is to identify age associated hematopoietic cell-intrinsic and cell-extrinsic factors that determine initiation and progression of MPN at young versus old age in mouse models carrying a JAK2-V617F or JAK2-V617F mutant allele.

Methods: To assess the effect of aging on MPN initiation and progression we studied the young and aged inducible transgenic mouse models of MPN. Integromics analysis was performed on MPN initiating stem and progenitor cells. Whole genome bisulfite sequencing was carried out in aged mice. The mutation profiles in patients with pediatric MPN appear to be less complex than in older MPN patients. We are currently investigating the relative contributions and collaborations of age-associated cell intrinsic and extrinsic changes in HSCP and BM niche in the course and severity of MPN in mouse models carrying a JAK2-V617F mutation, and in naturally aged donors and recipients of bone marrow transplantations.

Summary/Conclusions: Our study provided novel molecular and cellular mechanisms underlying increased incidence of MPN manifestation in old age. The implications of this work goes beyond the MPN malignancy and the comprehension of data sets generated in study will serve as a model to the wider scientific community to study other types of malignancies. This knowledge ultimately will help to define novel strategies to delay or target the onset of MPN in an aging individual.
Results: At BL, 59% of pts had anemia (hb <10 g/dL); pts with BL anemia were more likely at BL to have platelet count <50,000/µL (51% vs 38%), and hemoglobin MF (71% vs 57%), and high DIPSS score (41% vs 14%). For those with BL anemia regardless of whether RBC transfusion-dependent (TD), PAC did not worsen hgb levels and the rate of clinical improvement in hgb was higher for pts in the PAC BID arm (25%) vs PAC QD (13%) or BAT (12%) arms (Table). For those with non-TD AEs occurring in 13 pts (34%) followed by fatigue in 12 (32%). Treatment interruptions due to AEs occurred in 19%, 15%, and 16% of pts treated with PAC QD, PAC BID, and RUX, respectively; 26 (68%) were intermediate-2 risk and 9 (24%) high-risk according to the DIPSS (Passamonti et al, Blood 2010). Median time on treatment was 73 years (range, 49-83); 19 pts (50%) previously received hydroxyurea, RUX, and/or corticosteroids. Median hemoglobin (Hb) level at study entry was 8.6 g/dL (range, 5.4-11.7); 11 pts (29%) were RBC-transfusion-dependent. Median spleen size by ultrasound was 17.9 cm (range, 12.6 - 28). At baseline, 30 pts (79%) had constitutional symptoms. Mutations of JAK2, MPL, and/or CALR were present in 28 (74%), 31 (8%), and 31 (8%) pts, respectively; 26 (68%) were intermediate-2 risk and 9 (24%) high-risk according to the DIPSS (Passamonti et al, Blood 2010). Median time on treatment was 12 cycles (range, 2-33). In total, 881 adverse events (AE) CTCAE 1-5 were recorded. Worsening of anemia within the first 6 cycles was the most frequent symptom. 8 pts had grade 3 anemia, 5 pts had grade 4 anemia (fatigue), and thrombocytopenia (n=6) were the most common toxicities. Thrombocytopenia (n=6) was the most common AEs occurred in 13 pts (34%) of which 5 were fatal (cardiac decompensation, pulmonary edema, infection, and complications due to anemia). 6 pts (16%) stayed on treatment for >24 cycles. Summary/Conclusions: In our study in advanced MF, combination of POM plus RUX was feasible with an objective response rate of 34%. Approximately one third of pts was treated beyond cycle 12 due to sustained therapeutic benefit. The overall profile and results from our MPNSG-0109 trial, a step-wise increase of the POM dosage is included for the 2nd study cohort to further improve anemia response. P700 COMBINATION THERAPY OF POMALIDOMIDE PLUS RUXITLINIB IN MYELOFIBROSIS: RESULTS FROM COHORT 1 OF THE MPNSG-0212 TRIAL (NCT01644110) F. Steigemann1, M. Grieshammer2, S. Koschmieder3, A. Reiter4, A. Hochhaus5, F. Heidel5, N. von Bubnoff6, T. Kindler7, H. Hebart8, M. Bangerter9, C. Harrison1, J. Mascarenhas2, R. Hoffman3, T. Galpaz4, B. Stein5, V. Gupta6, A. Szoke7, M. Drummond8, A. Pristupa9, T. Granston10, R. Daly10, S. Al-Fayoumi10, J.A. Callahan10, J.W. Singer10, J. Golub10, C. Jamieson12, R. Mesa13, S. Verstovsek14

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Background: Therapeutic options to address anemia in patients (pts) with Myelofibrosis (MF) are limited. In our MPNSG-0109 trial investigating pomalidomide (POM) in MF with cytopenia, anemia was improved in 14-29% of pts treated with POM 3.5-2.5mg POM once daily (QD) (Schlenk RF, Steigemann F et al. Leukemia 2016). Aims: To evaluate synergistic effects of POM plus ruxolitinib (RUX), we are currently investigating the combination therapy within the MPNSG-0212 trial (NCT01644110). Methods: MPNSG-0212 is designed as multicenter, single-arm phase-IIb trial with a target population of 38 pts in the first cohort. Primary endpoints are response rate after 12 cycles (28 days each) according to IWG-MRT (Tefferi et al., Leuk Res 2011). Secondary endpoints are safety, quality of life, progression-free, and overall survival. Main inclusion criterion is MF with anemia with PAC or BAT (Table) were in pts with BL hgb <10g/dL. Dose modifications or discontinuations due to anemia were uncommon (Table). No exposure-response relationship was evident for grade ≥2 TE anemia. Summary/Conclusions: In pts with MF and BL thrombocytopenia, PAC treatment led to clinical improvement in hgb and reduction of RBC transfusion needs vs BAT. Serious anemia, and dose modifications due to anemia were uncommon. PAC provides a treatment option for pts with MF, including those with BL thrombocytopenia and anemia, for whom available options are limited. P701 PACRITINIB (PAC) VS BEST AVAILABLE THERAPY (BAT), IN PATIENTS WITH MYELOFIBROSIS (MF) AND BASELINE (BL) THROMBOCYTOPENIA: FOCUS ON RUXITLINIB (RUX)-TREATED PATIENTS IN THE PHASE 3 PERSIST-2 TRIAL C. Harrison1, J. Mascarenhas2, R. Hoffman3, T. Galpaz4, B. Stein5, V. Gupta6, A. Szoke7, M. Drummond8, A. Pristupa9, T. Granston10, R. Daly10, S. Al-Fayoumi10, J.A. Callahan10, J.W. Singer10, J. Golub10, C. Jamieson12, R. Mesa13, S. Verstovsek14

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Background: MF is a life-threatening hematologic malignancy characterized by symptomatology, debilitating constitutional symptoms, and progressive cytopenias (anemia and thrombocytopenia). Currently, JAK1 inhibitor RUX is the only approved therapy for pts with MF. Although RUX has been shown to reduce splenomegaly and symptoms in pts with MF, it is associated with dose-limiting cytopenias and is not indicated for pts with platelets <50,000/µL. PAC is an oral kinase inhibitor with specificity for JAK2,FLT3, IRAK1, and CSF1R. In the phase 3 PERSIST-2 study of PAC vs BAT (including RUX) in pts with MF and BL thrombocytopenia, PAC was significantly more effective in terms of spleen volume reduction (SVR; P=0.001) and appeared to have a better benefit/risk profile vs BAT. Aims: This analysis examines outcomes for pts with MF treated with RUX in the phase 3 PERSIST-2 study. Methods: Pts with MF and BL platelet count ≤100,000/µL were randomized (N=511) 1:1:1 to PAC 400mg once-daily (QD), PAC 200mg twice-daily (BID), or BAT. BAT included any physician-selected treatments for MF, as well as symptomatic and/or symptom-directed treatment. The co-primary endpoints were the rates of pts achieving ≥35% SVR (by MRI/CT) and ≥50% reduction in total symptom score (TSS; MPN-SAF TSS 2.0) at week 24. Efficacy analyses used the intent-to-treat efficacy (ITT-E) population, which included all pts with randomization date allowing them to contribute data for a week 24 endpoint. Crossover from BAT to PAC was allowed after week 24 or splenic progression. Results: RUX was the most commonly received active BAT. 44 (45%) BAT pts received RUX (Figure) and 32 (33%) received only RUX. Of the 44 pts who received RUX on study, 17 (39%) had BL platelet counts <50,000/µL and would not have been candidates for RUX by approved indication (or study protocol)). PAC achieved higher rates of primary endpoints vs BAT (including RUX) pts treated with RUX on study. One (3%) and 6 (19%) RUX pts (n=32 in ITT-E population achieved SVR ≥35% and TSS reduction ≥50% at week 24, respectively, vs 11 (15%) and 13 (17%) PAC QD and 16 (22%) and 24 (32%) PAC BID pts. For PAC pts with prior RUX, SVR and TSS endpoints were achieved in 6% and 10% with QD, and 13% and 32% with BID, vs 20% and 23% with QD, and 28% and 33% with BID for PAC pts without prior RUX. Grade 3/4 adverse events (AEs) were reported in 76%, 70%, and 45% of PAC QD, PAC BID, and RUX pts, most commonly (59% in any arm) thrombocytopenia (31%, 22%, anemia (27%, 22%), and neutropenia (9%, 7%) with PAC QD, BID, and RUX, respectively. Half (22/44) of RUX-treated pts crossed over to PAC treatment, at a median of 26.1 (95% CI 25.3-27.3) weeks. Of those 22, 19 pts remained on PAC treatment at the time of data cut-off, 7 for 24 weeks of PAC treatment (Figure 1).
SAFETY AND EFFICACY OF RUXOLITINIB (RUX) IN ELDERLY PATIENTS (≥75 YEARS) WITH MYELOFIBROSIS (MF): AN ANALYSIS FROM THE PHASE 3B, EXPANDED-ACCESS JUMP STUDY


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Background: RUX is a potent JAK1/JAK2 inhibitor that has led to reductions in splenomegaly and symptoms in patients (pts) with MF. Although few studies have assessed RUX in elderly pts, a recent analysis including 100 pts ≥75 y showed that RUX was safe and effective in these pts, with safety and efficacy outcomes similar to those in younger pts (Latagliata et al. Blood 2016;128:4251). JUMP, a large (N=2233), phase 3b, expanded-access trial assessed safety and efficacy of RUX in pts with no access to RUX outside a clinical trial and included a cohort of pts ≥75 y.

Aims: To assess the safety and efficacy of RUX in pts aged ≥75 y.

Methods: Pts with high- or Int-2-MF, or Int-1-risk pts with a palpable (≥25 cm) spleen, were eligible. RUX starting doses were based on baseline platelet (PLT) counts (5mg bid [≥50 to <100×109/L], 15mg bid [100 to 200×109/L], or 20mg bid [≥200×109/L]). Pts were ≥18 y; there was no maximum age limit. The primary endpoint was safety and tolerability of RUX. Secondary endpoints included changes in spleen length and symptoms.

Results: This analysis includes 416 pts (primary MF, 66%) who were ≥75 y and started treatment ≥1 y before data cutoff (01 Jan 2016). Baseline characteristics (median) were age, 78 y (range, 75-89 y); male, 57%; spleen length, 10 cm (0-35 cm); blast count ≥1%, 30.3%; hemoglobin, 101 g/L (<100 g/L, 46.9%); PLT count, 249×10^9/L (<100×10^9/L, 6.3%); ECOG PS =2, 84.9%. At data cutoff, more than half of pts remained on treatment or completed treatment per protocol (52.6%). As expected, a greater proportion of elderly pts discontinued RUX due to adverse events (AEs; 23.6%) or death (8.7%) than pts in the overall study (17.7% and 4.1%, respectively). Overall, 72.4% of pts had dose modifications (AEs, 58.4%), and 33.9% had an interruption (AEs, 31.5%). Safety of RUX in elderly pts was consistent with that in the overall population. Median exposure was 11 mo; mean average daily dose was 26.8mg (SD, 10.6). The most common hematologic grade 3/4 AEs were anemia (43.8%); overall, 34.1% and thrombocytopenia (22.1%: overall, 16.3%), AEs (all grade [grade 3/4]) in ≥10% of pts included ashenia (16.3% [2.6%]), pyrexia (18.0% [2.6%]), dyspnea (14.4% [4.3%]), diarrhea (13.5% [1.9%]), fatigue (11.8% [2.4%]), peripheral edema (10.8% [0.2%]), and pneumonia (10.1% [7.2%]). Infections in ≥5% of pts included pneumonia (10.1%), urinary tract infection (7.0%), and bronchitis (5.1%). Herpes zoster occurred in 3.9% of pts. At wk 24, 56.4% (124/220) of pts had a ≥50% reduction from baseline in spleen length (overall, 56.6%), and 19.1% (42/220) had ≥25%-50% reductions (overall, 23.3%); rates were similar at wk 48 (54.6% [65/119] and 19.3% [23/119]; overall, 61.6% and 18.9%). Most pts (64.2%) achieved ≥50% reduction at any time (Figure 1), similar to the overall population (70.2%). Pts also experienced significant improvements in symptoms. From wk 4 to 48, 42%-48% and 50%-57% of pts achieved a clinically meaningful response on the FACT-Lym TS and FACT-Fatigue, respectively.

Figure 1.

Summary/Conclusions: This analysis included the largest cohort of elderly pts with MF treated with RUX to date. Consistent with the study by Latagliata et al. RUX was safe and effective in pts ≥75 y, with pts achieving reductions in splenomegaly and symptoms similar to those in the overall population, with comparable rates of AEs. Additionally, findings from our study were consistent with those of the COMFORT studies, which included few pts ≥75 y. Overall, our study provides further evidence that RUX is safe and effective in elderly pts with MF.

P703

PROGNOSTIC RISK MODELS FOR TRANSPLANT DECISION-MAKING IN MYELOFIBROSIS


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Summary/Conclusions: This phase 3 PERSIST-2 study of PAC vs BAT in pts with MF and BL thrombocytopenia, although 19% of RUX-treated pts achieved a 50% reduction in TSS, RUX-treated pts rarely achieved SVR ≥35% at week 24. Rates of grade 3/4 AEs were higher with PAC vs RUX treatment, though the majority of RUX-treated pts began with 5mg dosing. Rates of dose reductions and discontinuations due to AEs with PAC BID and RUX were similar. Following crossover to PAC in 22 RUX-treated pts, 19 remained on treatment at the time of data cut-off.
Background: Accurate disease risk stratification is crucial for transplant decision-making in high-risk myelofibrosis (MF). A limited number of prognostic models are available, it is unknown if they are equivalent in the way they distribute patients into risk groups and in their discriminatory power to predict survival.

Aims: We have compared the performance of the International Prognostic Scoring System (IPSS), dynamic IPSS (DIPSS), DIPSS-plus, and Rumi’s score in a series of 544 MF patients aged 70 years or younger at time of diagnosis.

Methods: The Spanish Registry of Myelofibrosis is a nationwide, longitudinal registry contributed by centers associated to the Grupo Español de Enfermedades Mieloproliferativas (GEMFIN). From January 2000 to January 2016, a total of 544 adult patients aged ≤ 70 years with primary MF (n=335) or secondary MF (n=209) had been included in the registry. Cases of the prefibrotic form of MF were not considered. Comparison of the relative power of each prognostic model to discriminate levels of risk was estimated by means of the Harrell’s concordance index (C-index) and the $R^2$ explained variation. All the statistical analyses were performed with IBM SPSS 22.0 and Stata 11.

Results: In intermediate-2 disease, survival from diagnosis to intermediate MF was 3.35 years, 177 patients (33%) had died, and the remaining were censored alive. Sixty-nine patients (13%) had been submitted to allogeneic stem cell transplantation, after a median time of 20 months from MF diagnosis. The median projected survival of the overall series was 9.46 years (95% confidence interval: 7.44-11.30) and only reached for the low risk category of all classifications (and Rumi’s very low risk category). The projected survival for patients in the intermediate-1 group (intermediate in the Rumi’s score) and in the high-risk group (very high risk in the Rumi’s score) was comparable in the four models. By contrast, the Rumi’s high risk group had a projected median survival of 9.2 years, whereas that of the intermediate-2 categories by the IPSS, DIPSS, and DIPSS-plus models was 6.6 years, 5.6 years, and 6.5 years, respectively. The number of patients in the intermediate-2 and high risk categories was smaller in the DIPSS than in the IPSS or the DIPSS-plus. Overall, the Rumi’s score yielded the highest power to discriminate between risk categories as measured by the C-index and the $R^2$ explained variation. However, the IPSS and DIPSS-plus were the best models to discriminate between the intermediate-1 and intermediate-2 risk categories, which is the critical cut-off point for patient selection to transplant.

Summary/Conclusions: In our contemporary series of MF patients only the high-risk category is currently the only prognostication system that discriminate for patients in the intermediate-1 group (intermediate in the Rumi’s score) and in the high-risk group (very high risk in the Rumi’s score) was comparable in the four models. By contrast, the Rumi’s high risk group had a projected median survival of 9.2 years, whereas that of the intermediate-2 categories by the IPSS, DIPSS, and DIPSS-plus models was 6.6 years, 5.6 years, and 6.5 years, respectively. The number of patients in the intermediate-2 and high risk categories was smaller in the DIPSS than in the IPSS or the DIPSS-plus. Overall, the Rumi’s score yielded the highest power to discriminate between risk categories as measured by the C-index and the $R^2$ explained variation. However, the IPSS and DIPSS-plus were the best models to discriminate between the intermediate-1 and intermediate-2 risk categories, which is the critical cut-off point for patient selection to transplant. Patient selection for transplant is quite dependent on which prognostication model is used for disease risk stratification.

P704

LEUKEMIC TRANSFORMATION AND SECOND CANCERS IN 3649 HIGH RISK ET PATIENTS IN THE EXELS STUDY

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Background: A common therapy for essential thrombocythemia (ET), hydroxyurea (HU), has mutagenic properties and there is potential for leukemogenicity and secondary cancers with this agent. In the EXELS study, we report higher event rates for acute myeloid leukemia (AML) and other malignancies in HC-treated patients compared with those treated with anagrelide (ANA). However, there were large age differences between groups. Here, we present an updated analysis of the Harrell’s c-index and the $R^2$ explained variation. All the statistical analyses were performed with IBM SPSS 22.0 and Stata 11.

Methods: To assess the risk of AML and non-hematological malignancies in HC-treated patients compared with those treated with anagrelide (ANA). The caution advocated in the use of HC seems well advised.

Results: The number of AML cases was much lower (n=3) in patients who switched from HC to ANA versus the expected 0.22 cases. The number of AML cases was much lower (n=3) in patients who switched from HC to ANA the SIR was approximately 90-fold higher, with 20 AML cases observed versus the expected 0.22 cases. The number of AML cases was much lower (n=3) in patients who switched from ANA to HC. It has been proposed that exposure of ET patients failing HC therapy to a second potentially leukemogenic agent should be avoided; yet these data suggest that even after switching to a non-leukemogenic agent, HC-treated patients still have an over 90-fold increased risk of AML. Our data reinforces concerns of a leukemogenic effect of HC, with a markedly higher risk in patients failing HC and switching to another drug, even if that drug is ANA. The caution advocated in the use of HC seems well advised.

Summary/Conclusions: ET patients have a substantially increased risk for AML development; the SIR for AML was 40-fold higher than expected with HC exposure. In patients who switched from HC to ANA the SIR was approximately 90-fold higher, with 20 AML cases observed versus the expected 0.22 cases. The number of AML cases was much lower (n=3) in patients who switched from ANA to HC. It has been proposed that exposure of ET patients failing HC therapy to a second potentially leukemogenic agent should be avoided; yet these data suggest that even after switching to a non-leukemogenic agent, HC-treated patients still have an over 90-fold increased risk of AML. Our data reinforces concerns of a leukemogenic effect of HC, with a markedly higher risk in patients failing HC and switching to another drug, even if that drug is ANA. The caution advocated in the use of HC seems well advised.

P705

EPIDEMIOLOGY, OUTCOME AND RISK FACTORS FOR INFECTION COMPLICATIONS IN MF PATIENTS RECEIVING RUXOLITINIB. A MULTICENTER STUDY ON 373 PATIENTS


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Background: Infectious complications represent one of the most frequent cause of morbidity and mortality in Myelofibrosis (MF), the most severe of myeloproliferative neoplasms. Ruxolitinib (RUX), the first approved JAK1/2 inhibitor, significantly ameliorates disease-related splenomegaly and constitutional symptoms. Prospective controlled studies observed a high rate of infectious complications including opportunistic and unusual infections, probably due to its immune-suppressant activity. However, risk factors for infections in MF patients (pts) treated with RUX are still to be investigated.

Aims: To evaluate characteristics, incidence and risk factors for infections in RUX-exposed MF pts.

Methods: Clinical and laboratory data of MF pts treated with RUX were retrospectively collected from the database of 21 Italian Hematology Centers. Infections were defined according to the CTCAE.

Results: Overall, 373 pts received RUX between June 2011 and June 2016. At RUX start the clinical features were (median): age 68 years (27-89), >65y, 62%; male, 57%; Hb, 10.8g/dL (7-16.7); Plt <10g/dL, 40%; Plt 246×10^9/L (33-1887); Plt 100×10^9/L, 10%; spleen enlargement, 97%; spleen length ≥10cm, 66% constitutional symptoms, 52%. International Prognostic Score System (IPSS) was intermediate-1 (15%), intermediate-2 (46%), high (39%). JAK2V617F mutation was detected in 255 out of 313 evaluated pts (81%). Karyotype was unfavorable in 15 out of 203 evaluable pts (7%). Previous infectious complications were recorded in 31 pts (8%). After a median RUX exposure of 20 months (range, 1-56), 101 pts (27%) experienced 129 infectious events (grade 3, 33%), for an incidence rate of 14.9 cases for 100 pts/year. The rate of infections tended to decrease over time: 54% occurred within 6 months of RUX start, 40% between 6 and 12 months, 9% between 12 and 18 months (³grade 3, 33%), for an incidence rate of 14.9 cases for 100 pts/year. The rate of infections tended to decrease over time: 54% occurred within 6 months of therapy, 5% between 6 and 12 months, 9% between 12 and 18 months (p=0.0001). Respiratory tract infections were more frequently observed (73 events, 57%). Cutaneous, urinary tract and gastrointestinal infectious events were diagnosed in 15%, 10% and 7% of cases, respectively. In 14 cases fever of unknown origin was recorded (Figure 1). Etiological agents were isolated in 14 cases (11%); bacteria in 9 cases (gram+ 56%, gram- 22%, C. difficile diarrhoea 22%) and fungi in 2 cases (pulmonary aspergillosis and oesophageal candidiasis). Mycobacterium tuberculosis M. avium was isolated in 3 cases. Herpes-virus reactivations occurred in 12 cases (9%). No patients reactivated hepatitis B virus. At last follow-up, 88 pts (24%) have died, in 10 cases (11%) due to infectious complication. Among baseline features, age≥65 years at RUX start (p<0.0001), previous infection (p=0.001), primary vs secondary MF (p=0.021) and high IPSS (p=0.029) significantly correlated with higher infectious risk. Notably, no differences were observed according presence of large (≥10cm) splenomegaly, higher (≥20) total symptoms score, presence of cytopenias, Charlson comorbidity index (>2) and body mass index (>21 and >30). In multivariate analysis, PMF diagnosis (HR 1.6 CI95% 1.07-2.5), age≥65 years (HR 2.1 CI95% 1.3-3.3) and previous infection (HR 3 CI95% 1.7-5.4%) confirmed their negative prognostic association. Interestingly, RUX dosage, spleen response and hematological toxicities during treatment were not associated with infectious risk.

Summary/Conclusions: Infections occurred in around one-third of RUX-treated pts; the rate of infections tended to decrease over time, and were fatal in 11% of the cases. Advanced age, a previous infectious event and diagnosis of PMF seem to be the main contributors to infectious risk.
ET) chose watchful waiting to manage >25% of their pts at diagnosis; 22% of untreated pts had moderate to high (quartiles 3-4) overall symptom burden. Physicians primarily recommended treatment for pts experiencing severe symptoms (72% MF, 68% PV, 72% ET) or symptomatic splenomegaly (71% MF, 61% PV, 39% ET). PLB was mainly used to treat pts with PV. Of those who received PLB (n=155), 71% were very or somewhat satisfied; 25% were very satisfied and felt that PLB had a negative impact on their QOL. Similarly, 37% of physicians felt that PLB had a negative impact on pt QOL; PLB alone was insufficient for disease control in 38% of pts. Pts stopped PLB because physician deemed it no longer necessary (62%), pts felt worse after treatment (10%), and visit frequency was inconvenient (8%). Physician-reported reasons for stopping PLB were that visit frequency was inconvenient (38%), pts felt worse after treatment (35%), and lack of intravenous access (33%). HU use was assessed in pts with PV or ET. Of those who received HU (PV, n=95; ET, n=145), 78% and 74%, respectively, continued to receive HU; 19% and 22% were dissatisfied with HU therapy. Main reasons for stopping HU were lack of efficacy (29%, PV, 13% ET) and toxicity (10% PV, 27% ET). Overall, 78% of physicians reported that up to 25% of their patients showed inadequate efficacy or intolerance of HU. Main measures of treatment success among pts were physician feedback (73% MF, 75% PV, 75% ET) and blood counts (72% MF, 67% PV, 74% ET). Lack of efficacy, side effects, and discontinuation were key reasons for changing treatment.

Summary/Conclusions: Many pts with MPNs are managed with watchful waiting at diagnosis. Although most of these pts have a low symptom burden, 22% have a moderate to high burden, highlighting the need for proactive and standardized symptom assessments at diagnosis and over the course of treatment. Intensive cooperation of physicians and pts felt that phlebotomy had a high negative impact on pt QOL. Overall, pts consider physician feedback and blood counts to be important indicators of treatment success.

P707
SUCCESSFUL LONG-TERM MAINTENANCE OF PV PATIENTS WITH A MONTHLY SCHEDULE OF ROPEGINTERFON ALFA-2B: AN UPDATE FROM THE PEGINVERA STUDY

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Background: The outcome of patients with Philadelphia negative myeloproliferative neoplasms (MPN) who transform to acute leukemia is abysmal. There have been no advances in targeted therapy for this cohort of patients or individualized treatment based on genomic information. Furthermore, no large studies have investigated the impact of molecular profiling on clinical outcome in patients with accelerated or blast phase of MPN.

Aims: To describe the clinical outcomes of patients with MPN who transform to accelerated or blast phase and evaluate the impact of genomic alterations on outcomes.

Methods: Eligibility criteria included: Prior diagnosis of Philadelphia negative MPN according to WHO 2008 criteria; evidence of transformation to accelerated (10-19% blasts in peripheral blood or bone marrow) or blast phase (≥20% blasts) and seen at Princess Margaret Cancer Center between January 1998 and February 2017. The primary endpoint was overall survival (OS); defined as the time from transformation to death or last follow-up. Secondary endpoints included survival based on curative versus non-curative approach and treatment over time. In addition the impact of mutations will be correlated with clinical outcomes and survival.

Figure 1. Results: One hundred and eighty-seven patients who transformed to accelerated or blast phase with a prior diagnosis of MPN were identified at our insti-
Other Non-malignant hematopoietic disorders

P709

MASITINIB FOR TREATMENT OF SEVERELY SYMPTOMATIC INDOLENT SYSTEMIC MASTOCYTOSIS: ADDITIONAL EFFICACY ANALYSES FROM THE RANDOMIZED, PLACEBO-CONTROLLED, PHASE 3 STUDY AB06006. 

Background: Masitinib, a selective oral tyrosine kinase inhibitor targeting wild-type KIT, LYN and FYN, was the first drug to demonstrate efficacy in a phase 3 setting (study AB06006) for treatment of patients with severe indolent systemic mastocytosis (ISM) who are unresponsive to existing, optimal symptomatic treatments. In The Lancet (Feb 11:389(10069):612-620), Lortholary and colleagues reported a significant and clinically meaningful treatment benefit for masitinib (6mg/kg/day over 24-weeks) versus placebo, with primary analysis based on cumulative response (≥75% improvement from baseline, timeframe weeks 8-24, comprising 5 visits at 4-week intervals) in at least one of four severe baseline symptoms (pruritus, flushes, depression, or fatigue) using repeated measures methodology for rare diseases (i.e. a longitudinal analysis with respect to symptoms as opposed to patient response rate at a single point in time). Eligible patients were aged 18–75 years and had ISM according to inclusion criteria that were slightly broader than the WHO classification.

Aims: To aide interpretation of this study's prospectively declared primary endpoint via comparison with additional efficacy analyses based on a cohort restricted to the WHO classification of ISM and more conventional patient-centric response endpoints.

Methods: Randomized, placebo-controlled, phase 3 study that included 135 severely symptomatic ISM patients, including the subvariant smoldering systemic mastocytosis (71 masitinib, 64 placebo), 80% of whom satisfied the WHO classification.

Results: Masitinib showed a significant improvement over placebo according to its pre-specified primary endpoint (mITT population), with a cumulative response of 18.7% versus 7.4%, respectively, odds ratio (OR) of 3.6 [95%CI 1.2-10.8], P=0.008 (with re-randomization). This outcome was confirmed in the WHO patient subgroup: 17.8% versus 8.0%, respectively, OR=3.25 [0.97-10.88], P=0.0317. Computing the primary analysis (mITT) according to cumulative response per patient (GEE model) was also positive: 26.7% versus 12.8%, respectively, OR=2.48 [1.16–5.31], P=0.0212, as was analysis according to individual patient response (Pearson chi-square): 40.3% versus 24.2%, respectively, P=0.0062. Response (per patient) on all severe baseline symptoms for at least one visit was: 16.4% versus 1.6%, respectively, P=0.0062. Finally, analysis of sustained response in all severe baseline symptoms over multiple visits was highly discriminatory between treatment-arms: for patients with 3 severe baseline symptoms, masitinib generated a 12.5% response rate (≥75% improvement in each symptom) for 3 out of 5 visits, versus no response for placebo; and for patients with 2 severe baseline symptoms masitinib generated a response rate of 21.1%, 15.8% and 10.5% over at least 1, 2, and 3 visits, respectively, versus no response for placebo.

Summary/Conclusions: These post-hoc analyses confirm the clinical relevance, durability, and generalizability of the positive primary endpoint from study AB06006. Findings therefore support the conclusion that masitinib generates a significant therapeutic benefit in patients with severely symptomatic ISM who were unresponsive to optimal symptomatic treatments.

P710

THERAPY RESPONSE AND LONG-TERM OUTCOME OF 71 ADULT PATIENTS WITH HEMATOLOGICAL MALIGNANCY-ASSOCIATED HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS: A SINGLE INSTITUTION EXPERIENCE

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Background: Hematological malignancies (HMs) often generate an inflammatory reaction resulting in hypofibrinogenemia, hyperferritinemia, and elevated LDH. Paraneoplastic hemophagocytic lymphohistiocytosis (HLH) is a potentially fatal, immune-mediated, systemic disorder characterized by fever, pancytopenia, and hemophagocytosis in the bone marrow. A diagnostic criterion for classical HLH is a rise in ferritin levels to above 500 μg/L. Despite advances in systemic therapies and supportive care, HLH remains an immune reaction to HMs that can be life-threatening, with high mortality rates. The phase 3 randomized, placebo-controlled, phase 3 study AB06006 (Haematologica | 2017; 102(s2) | 285)
had HLH solely attributed to malignancy (Figure 1).

Thirty-four patients (32%) developed HLH from solid malignancies and 29 patients from hematological malignancies. Fifty-four (76%) patients had lymphoid malignancy and 42 patients myeloid malignancy.

Results: Seventy-one adults, aged 22–84 years, were diagnosed with aggressive hM-HLH during the 8-year period. Lymphohistiocytosis was diagnosed in 85 patients and myeloid malignancy in 29 patients. Fifty-four (76%) patients with hM-HLH were prospectively collected. Review concerned records of 142 adults with suspected HLH, hospitalized between Jan 2009 and Dec 2016. Of those, 71 patients with hematological malignancy were diagnosed with HLH and included to the present study. Hematological malignancy was defined as a neoplasm of lymphoid or myeloid origin. In all studied patients, the diagnosis of HLH was based on the HLH-2004 criteria. Infection as a possible additional trigger of HLH was carefully studied in all our hM-HLH patients. EBV and CMV DNA were routinely examined in whole blood, using RT quantitative PCR; other viruses (e.g. adenovirus, HSV, VZV, HHV6, influenza) were studied based on indications. Blood and urine cultures were performed in order to reveal any bacterial or fungal infections. Tests for fungal antigens, tuberculosis, and parasites were also performed if indicated. HLH treatment categories have included proapoptotic chemotherapy (etoposide, corticosteroids, IVIG) and T cells (corticosteroids, cyclosporine A).

Summary/Conclusions: HLH in the context of malignancy is still considered a challenging adult hematologic malignancy. It is a highly lethal disorder in adults. The patients who develop hM-HLH with concomitant infection during chemotherapy show better survival than those who had HLH solely attributed to malignancy. Although poor outcome in some patients with M-HLH is related to malignancy progression, in some patients the lack of effective M-HLH therapy may further impede adequate treatment of malignancy.

Methods: From 2008 and onwards, data on adult patients referred to the Hematology Center Karolinska with suspected HLH were prospectively collected. Review concerned records of 142 adults with suspected HLH, hospitalized between Jan 2009 and Dec 2016. Of those, 71 patients with hematological malignancy were diagnosed with HLH and included to the present study. Hematological malignancy was defined as a neoplasm of lymphoid or myeloid origin. In all studied patients, the diagnosis of HLH was based on the HLH-2004 criteria. Infection as a possible additional trigger of HLH was carefully studied in all our hM-HLH patients. EBV and CMV DNA were routinely examined in whole blood, using RT quantitative PCR; other viruses (e.g. adenovirus, HSV, VZV, HHV6, influenza) were studied based on indications. Blood and urine cultures were performed in order to reveal any bacterial or fungal infections. Tests for fungal antigens, tuberculosis, and parasites were also performed if indicated. HLH treatment categories have included proapoptotic chemotherapy (etoposide, corticosteroids, IVIG) and T cells (corticosteroids, cyclosporine A).

Figure 1.

Results: Seventy-one adults, aged 22–84 years, were diagnosed with aggressive hM-HLH during the 8-year period. Lymphohistiocytosis was diagnosed in 42 patients and myeloid malignancy in 29 patients. Fifty-four (76%) patients developed HLH as a first manifestation of unknown malignancy, during aggressive disease, or malignancy relapse. The remaining 24% patients developed HLH during chemotherapy. In 14 patients, HLH therapy started before confirmation of HLH diagnosis, based on suspicion of HLH (mean 6.7±8.4 days; median 2 days; range 1–31 days). Seventeen patients started HLH therapy at the day of HLH diagnosis. In 36 patients HLH therapy started after confirmation of HLH diagnosis (mean 15.9±14.3 days; median 5 days; range 1–242 days). Forty of 71 (56%) patients with active HLH died, of which 20 had signs of progressive malignancy. 16 patients had generalized infection (bacterial - 12 patients, viral - 3 patients, fungal - 4 patients; some patients had more than one) and 10 patients had concomitant central nervous system bleeding. Thirty-one (44%) patients responded to HLH therapy and achieved remission of HLH. However, only 13 of 71 (18%) patients with HM-HLH were still alive after a median follow-up time of 50 months, despite the attempted treatment in 67 (94%) cases. The probability of overall survival (OS) from 6, 12, 24 and 60 months after HLH diagnosis was 39, 20, 15 and 15%, respectively. The patients who developed HM-HLH with concomitant infection during chemotherapy had significantly longer OS (p=0.03) compared to patients who had HLH solely attributed to malignancy (Figure 1).

Summary/Conclusions: HLH in the context of malignancy is still considered a challenging adult hematologic malignancy. It is a highly lethal disorder in adults. The patients who develop hM-HLH with concomitant infection during chemotherapy show better survival than those who had HLH solely attributed to malignancy. Although poor outcome in some patients with M-HLH is related to malignancy progression, in some patients the lack of effective M-HLH therapy may further impede adequate treatment of malignancy.
**Background:** Erythrocytoses are characterized by an elevated red cell mass. The most widely studied disease is Polycythemia Vera (PV), however, other types of erythrocytoses can be either inherited (Congenital Erythrocytosis-CE) or acquired (Erythrocytosis due to hypoxia or reactive erythrocytosis, known as secondary erythrocytosis related to lung, cardiac or renal disorder. Next generation sequencing (NGS) has been used to analyse the presence of mutations in 28 genes (enlarged hypoxia pathway and other candidate genes).

**Results:** To date, samples from 140 patients have been reported, among whom 46 have been tested using NGS approach. Variants in 14 patients (13 males and 1 female; median age 50 y. [12-71]) with unknown significance have been detected, including 4 in PHD genes, 5 in HIF genes, 4 in LNK genes (SH2B3) and 1 in JAK2 variant. In patients with variants, a familial history of erythrocytosis was noted in 3. No independent thrombotic complication was reported in the 15 patients. In 2 patients (one with a JAK2 variant and one with a JAK2K75H variant), the erythropoietin was low, whereas for the others, the erythropoietin was normal. Of note, the median age of the patients was surprisingly high, suggesting that the diagnosis was not previously performed due to the absence of available tests. Functional studies were performed on PHD2 variants: a significant decrease in the hydroxylase activity was noted for one variant, but not for the others. On the other hand, a decrease in the stability along time of the PHD2 protein was observed for two variants, underscoring the different mechanisms involved in the impairment of the PHD2 activity.

**Summary/Conclusions:** NGS is a useful tool to explore mutations in CE, but identifies genetic variants in only 30% of patients with such disorder. Further exams including whole exome sequencing are planned to achieve a right diagnosis in the 70% remaining CE patients. *In vitro, in cellulo and in vivo* (including zebrafish model) functional studies are currently performed to validate the clinical relevance of the variants identified in the hypoxia pathway. They are compared to variants identified in the development of tumors in order to dissect the molecular mechanisms of this finely tuned pathway.

**P713**

**CHARACTERIZATION OF CD34+ HEMATOPOIETIC PRECURSORS IN INDOLENT SYSTEMIC MASTOCYTOSIS AND THEIR POTENTIAL ROLE IN EARLY DISPERSION OF THE DISEASE**

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**Background:** Recent studies show that most systemic mastocytosis (SM) patients, including indolent SM (ISM) with (ISMw+) and without skin lesions (ISMw-), carry the KIT D816V mutation in PB leukocytes.

**Aims:** To investigate the potential association between the degree of involvement of BM hematopoiesis by the KIT D816V mutation and the distribution of different maturation-associated compartments of bone marrow (BM) and peripheral blood (PB) CD34+ hematopoietic precursors (HPC) in ISM, and identify the specific PB cell compartments that carry this mutation.

**Methods:** The distribution of different maturation-associated of BM and PB CD34+ HPC from 64 newly-diagnosed (KIT-mutated) ISM patients and 14 healthy controls was analyzed by flow cytometry. In 18 patients distinct FACS-purified PB cell compartments were also investigated for the KIT mutation.

**Results:** ISM patients showed higher percentages of both BM and PB KIT-mutated CD34+ HPC vs controls, particularly among ISM cases with MC-restricted KIT mutation (ISMwMC): this was associated with progressive blockade of maturation of CD34+ HPC to neutrophil lineage from ISMwMC to multilineage KIT-mutated cases (ISMwMC). Regarding the frequency of KIT-mutated cases and cell populations in PB, variable patterns were observed, the percentage of KIT-mutated PB CD34+ HPC, eosinophils, neutrophils, monocytes and T-cells increasing from ISMwMC to ISMwMC+ to ISMw patients.

**Summary/Conclusions:** Positivity for the KIT D816V mutation in PB of ISM is associated with (i) a restricted involvement of central (epithelial/myoid, dendritic, myeloid and lymphoid) and of multiple myeloid cell populations, KIT-mutated PB CD34+ HPC potentially contributing to disease dissemination already at very early stages.
Background: Erythrocytosis, (i.e. increased levels of hemoglobin /hematocrit (Hb/Htc) >95percentile for age and sex), is rarely found in pediatric or adolescent age. Presence of familial cases, presentation at birth or presence of known mutations, as well as exclusion of secondary causes identifies primary (PE) or congenital secondary forms (CE). However, many cases still lack evident etiological definition (idiopathic E.). Moreover, natural course and treatment are still anecdotally reported.

Aims: Here we present our experience in a large and heterogeneous series of children with absolute erythrocytosis. The aims is to identify a possible clinical and diagnostic approach to children with erythrocytosis.

Methods: All children with E. who lacked evidence of reactive origin were consecutively referred to our laboratory for molecular evaluation. Molecular analysis of the main involved genes (VHL, HIF2A, EPOR, JAK2, PHD2) was performed by allele specific PCR, PCR on direct DNA sequencing. Erythopoietic Colony Essay (ECC) was performed on peripheral blood with and without cytokines. Clinical features and treatment choices were reported by referring clinicians (table 1).

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Results: Patients were grouped according to the definitions of absolute Erythrocytosis. A total of 44 pediatric cases were identified (less than 18 years old). There were 7 families, where 5 adults were also found polyglobulic. However, in only 4 families a defect was identified (2 VHL, and 2 Hb variants). One high positive case was found sporadic. Most Hb variants were not symptomatoc, while all other familiar cases had splenomegaly and vascular symptoms. Among non familial, non genetic cases, 5 children were affected by Down Syndrome; 4 children had severe renal or cerebral disease. In one 4 year old girl, with a polymorphic VHL variant, who presented with arterial hypertension, a small size ganglionneuroma was found after a 5yrs follow-up. In 21 cases non causes could be identified. They were mostly male (n18; presented at adolescent age with advanced puberal status (n17; many were symptomatic (6). Only one 9 year old girl was diagnosed with Polycytemia vera (JAK2/V617F positive). Treatment varied according to physician decisions and presence of vascular symptoms, 6 children received ASA and 11 were phlebotomised. In two older patients severe vascular complications were observed (arterial thrombosis), even with Htc<45%

Summary/Conclusions: This series shows the heterogeneity of Erythrocytosis as found in pediatrics. Extensive clinical and genetic analysis are required but still a large number of cases lack clear definitions. The usefulness of antigaggregation and phlebotomy is not proved.

Pt716

NEUROLOGIC INVOLVEMENT IN EVANS SYNDROME AND CHRONIC HEMOLYTIC AUTOIMMUNE ANEMIA OF CHILDREN: DESCRIPTION, EVOLUTION AND GENETICS


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Summary/Conclusions: Patients have been given steroids (n=6), intravenous immunoglobulins (n=2) or immunosuppressive treatment (n=2) inflammatory lesions with hyperintense T2 signal in all patients, gadolinium-enhancing lesions in 7 and perilesional edema in 5. Five patients had a total of 8 biopsies, which confirmed the inflammatory process with macrophagic (n=3) or lymphoplasmocytic (n=5) infiltrates. In 4 cases, a lymphocytic meningitis was associated. Non-neurological organ involvement was present in all patients, mainly pulmonary nodules (n=6) and lymphoproliferation (n=4). All patients had an abnormal immunophenotype, with T-cell (n=7) or B-cell (n=3) deficiency and hypogammaglobulinemia was present in 7 of the 8 cases. Patients have been given standard treatment (n=6), immunosuppressive treatment (n=3), Ciclosporin, Mycophenolate Mofetil and Methylxate, improving symptomatic monitoring and MRI for all. Five patients relapsed and 3 patients had an asymptomatic radiological progression. At the last follow up point, all patients had neurological sequelae and 7 persisting radiological abnormalities. Four out of 6 patients analyzed had a PID: 22q11.2 microdeletion (n=1), heterozygous C7LA mutation (n=2) or homozygous LRBA mutation (n=1).

Summary/Conclusions: Neurological involvement is a rare and severe late event in the course of childhood ES, or exceptionally AHA, that may reveal various underlying PID. Complete imaging and pathology examination highlight a causative immune dysregulation and could guide specific therapeutic strategies.
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Background: The most frequent Autoimmune Neutropenia (AIN) in childhood is the primary type (p-AIN), whereas in adults it is mostly represented by secondary neutropenias, which can be associated to infection, drug administration, immunodeficiency, neoplasms, bone marrow transplantation or other autoimmune disorders.

Aims: To describe clinic and laboratory findings in children affected by AIN secondary to other autoimmune diseases (s-AIN).

Methods: This registry study analyzes 26 patients affected by s-AIN enrolled in the Italian neutropenia registry of A.I.E.O.P. (Associazione Italiana di Oncocoematoologia Pediatrica) over a 15-year time-span: this cohort, the largest ever described, was compared to 263 patients affected by p-AIN enrolled in the Registry in the same period.

Table 1.

Results: Specific characteristics of s-AIN patients are presented in Figure 1. The prevalence of former preterm babies among p-AIN (and not s-AIN) patients was significantly higher than in a cohort of 487 consecutively hospitalized children (p=0.0002). The median age of onset of AIN was 0.77 year and 10.07 year in p-AIN and s-AIN respectively (p=1.105e-12). The prevalence of selected IgA deficiency was 3% in p-AIN and 13.6% in s-AIN children: both prevalences were significantly higher than that (0.21%) of a group of 470 controls (p=0.0009 in p-AIN and p=7.239e-12 in s-AIN). Median value of neutrophils was lower in p-AIN (0.45 x 10^9/L) than in s-AIN 0.63 x 10^9/L (p=0.03); median value of lymphocytes was significantly reduced (p=6.29e-11) in s-AIN (1.58 x 10^9/L vs p-AIN (4.36 x 10^9/L) group. Leukopenia (p=1.80e-07) and severe infections (p=0.0001) occurred more frequently in s-AIN; monocytesis (p=0.039) and spontaneous remission (p=3.21e-11) in p-AIN. GCSF was used in 6.9% of the p-AIN and 13% of the s-AIN patients (p=0.0045). Neutropenia appeared contemporarily to other autoimmune manifestations in 11/26 s-AIN patients (42.3%), appeared firstly in 8/26 patients (30.7%) (median and mean time of appearance of other autoimmune signs: 440 and 987 days respectively) and later in 7/26 patients (26.9%) (median and mean time of appearance of s-AIN: 558.5 and 866.3 days respectively). Evans Syndrome (ES) and autoimmune thyroiditis (AT) were the most common secondary autoimmune diseases (11 and 7 patients, respectively), whereas 7 s-AIN patients presented not previously reported associations: 3 with GH deficiency, 2 with coeliac disease (CD), 1 with autoimmune hepatitis (AH) and 1 with autoimmune-encephalitis. In 6 children s-AIN was associated with more than one defined autoimmune disease and in 4 children with undefined autoimmune signs characterized by arthralgia and ANA positivity. Finally, only 2/26 patients presented spontaneous remission: a boy who recovered from ES and one patient, affected by both AT and CD who, after starting a gluten-free diet, recovered from s-AIN (and not from AT). A third girl suffering from both AH and bi-lineage ES (thrombocytopenia + AIN) has been maintained, 30 months after the stop therapy, a stable remission from AH and thrombocytopenia (but not from s-AIN).

Summary/Conclusions: p-AIN is in the vast majority of cases a benign and self-limiting disorder typically occurring under 2-3 years old whereas s-AIN is a more severe disease, usually appearing after the first 5 years of life, usually associated to lymphocytopenia and with a highly frequent tendency to become chronic.

P718 PAROXYSMAL NOCTURNAL HEMOGLOBINURIA TREATMENT DURING PREGNANCY

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a life-threatening disorder with a high risk of thrombosis. Targeted therapy radically changed the prognosis in PNH. Therefore issues of reproductive health in PNH patients are becoming very important. Recently the management of PNH during pregnancy has been challenging because of the high risk of maternal morbidity and frequent pregnancy loss. The combination of targeted therapy with eculizumab and anticoagulants made it possible not only to increase the survival rate, but also to improve the quality of life.

Aims: We compared the pregnancy outcomes in PNH patients on eculizumab treatment and retrospective data of pregnancies on symptomatic therapy only.

Methods: Since 1999 we have analyzed 32 pregnancies in PNH patients. 17 patients (group 1) from 2013 exposed to eculizumab during pregnancy with anticoagulants. Other 15 women (group 2) received only symptomatic therapy. The median of PNH granulocyte clone at that time was 74.7% (23-99). PNH diagnosed before the pregnancy in all cases. 64.3% of them had previously received immunosuppressive treatment of aplastic anemia. 18.7% patients registered venous thromboses before conception. 92.9% of patients had been using eculizumab prior to becoming pregnant, mean duration of therapy was 21 months (4-44). Anticoagulation with low molecular weight heparin was used in 85.7% pregnancies.

Results: Clinical manifestations of hemolysis significantly regressed during eculizumab treatment: normalization of LDH was registered in 76.5% patients. Without eculizumab LDH level increased in all pregnant patients. No maternal death and thrombotic events have been observed. 42.9% of patients required a dose adjustment due to breakthrough hemolysis (a dose increase and/or more frequent use of eculizumab). Pregnancy complications were less frequent with eculizumab: abortion threat 35.3% vs 85.7%, fetal growth retardation syndrome 7.1% vs 21.4%, preeclampsia 5.9% vs 14.3%. Transfusion rate was higher without eculizumab (86.7% vs 41.2%). Pregnancies resulted in the birth in 100% patients exposed eculizumab and 42.9% on supportive treatment. Mean birth weight 2560 g (450-3550). Most of newborns (87.5%) are healthy, 83.3% of them received breastfeeding without complications both on eculizumab and without it.

Summary/Conclusions: We can conclude that pregnancy outcomes in PNH patients with eculizumab are much better than with symptomatic therapy only. Our data demonstrate the possibility of safe therapy with eculizumab in pregnant women. Pregnancy does not worsen the prognosis of PNH in the case of targeted and adequate supportive therapy. There is no difference in health between infants born by mothers with PNH and the newborns from general population.
Platelet disorders: Clinical

P719
LONG-TERM RESPONSE TO ORAL ELIGLISUAT IN TREATMENT-NAÏVE ADULTS WITH GAUCHER DISEASE TYPE 1: FINAL EFFICACY AND SAFETY RESULTS FROM A PHASE 2 CLINICAL TRIAL AFTER 8 YEARS OF TREATMENT
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Background: In Gaucher disease type 1 (GD1), deficient lysosomal acid β-glucocerebrosidase activity leads to accumulation of glucosylceramide, primarily in macrophages (Gaucher cells), which deposit in the spleen, liver, and bone marrow, leading to thrombocytopenia, anemia, hepatosplenomegaly, and skeletal disease. Hematologists often identify and manage the disease. Intravenous enzyme replacement therapy (ERT) with recombiant acid β-glucosidase has been the mainstay of therapy for GD1. Eliglusuat is an oral substrate reduction therapy approved as first-line treatment for adults with GD1 with poor, intermediate, or extensive CYP2D6-metabolizer phenotypes (>90% of patients). Phase 3 trials demonstrated safety and efficacy of eliglusuat in naïve patients (Mistry et al. JAMA. 2015) and safety and stability in patients switching from long-term ERT (Cox et al. Blood. 2017). We report the final 8-year results of an open-label Phase 2 trial (NCT00358150, Sanofi Genzyme) in previously untreated adults with GD1. These data build on 1-, 2-, and 4-year data showing sustained improvements in hematologic parameters, organ volumes, disease-related biomarkers, and measures of bone health (Lukina et al. Blood Cells Mol Dis. 2014).


Methods: Adult GD1 patients who had splenomegaly with thrombocytopenia and/or anemia received 50 or 100mg eliglusuat片 rate (equivalent to 42 or 84mg eliglusuat) twice daily, dosed by plasma trough levels. Efficacy outcomes included changes in hemoglobin, platelets, spleen and liver volumes, disease-related biomarker levels, skeletal manifestations, and achievement of therapeutic goals for anemia, thrombocytopenia, splenomegaly, and hepatomegaly (Pastores et al. Semin Hematol. 2004; Lukina et al. Blood. 2010).

Results: Of 26 enrolled patients, 19 completed the trial and 7 withdrew: 2 on the first day of treatment due to asymptomatic nonsustained ventricular tachycardia detected during routine monitoring (plasma levels of eliglusuat were undetectable); 1 after 1 year due to progression of a bone lesion (retrospectively identified at baseline); 1 chose to withdraw after 2 years; and 3 due to pregnancy. Of 8 years of eliglusuat use, mean (±SD) hemoglobin level and platelet count increased by 2.1±1.7 g/dL (from 11.3±1.6 to 13.4±1.3 g/dL) and 110% (from 67.5±21.1 to 130.7±59.8 x109/L), respectively. Mean spleen and liver volumes (multiples of normal, MN) decreased by 68% (from 17.3±10.4 to 5.1±3.5 MN) and 31% (from 1.6±0.5 to 1.1±0.3 MN), respectively. All patients met ≥3 of 4 long-term therapeutic goals (spleen, 100% of patients; liver, 100%; hemoglobin, 93%; platelets, 53%) by 7-8 years. Median chitotriosidase levels decreased by 84%, C-reactive protein by 82%, and more than half of patients (59%) showed a normal (class 1) glucosylsphingosine (Lyso GL-1) level; plasma GL-1 normalized. Total mean lumbar spine bone mineral density increased by 0.12 g/cm2; mean Z-score increased by 0.88 (from -1.27±1.02 to -0.39±1.13) and mean T-score by 0.95 (from -1.64±1.07 to -0.69±1.31). Eliglusuat was well-tolerated. All quality of life measures (SF-36, fatigue severity score, disease severity score, disease activity score) showed improvement over time. Most adverse events in this long-term trial were mild or moderate in severity (98%, 342/348) and considered unrelated (94%, 328/348) to treatment.

Summary/Conclusions: After 8 years of treatment with eliglusuat, clinically meaningful improvements in hematologic, visceral, biomarker, and bone parameters continued or were maintained among patients in this Phase 2 trial. No new safety concerns emerged.

P720
REAL WORLD EVIDENCE ON DRUG UTILIZATION PATTERNS OF ELTROMBOGAP IN ADULT PATIENTS WITH IMMUNE THROMBOCYTOPENIA AND/OR IMMUNE THROMBOCYTOPENIA (ELTROMBOGAP) IN SELECTED COUNTRIES IN THE EUROPEAN UNION (EU) STUDY
E.O. Gutiérrez1,*, A. Salama2, J.-F. Viallard3, R.G. Delgado4, M.E. Mingote5, E. Avila Arreguin5, E. Quebe-Fehling5, O.A. Oelem6, A. Alpezzu7, T.J. González-López8,9,10,11
1Hospital Universitario Puerta de Hierro Majadahonda, Madrid, Spain, 2Charité Universitätsmedizin Berlin, Berlin, Germany, 3Hôpital Haut-Lévêque Centre FMagendie Avenue de Magellan, Bordeaux, France, 4Hospital Clínico Universitario Virgen de la Victoria Campus Universitario de Teatinos s/n, 5Hospital Regional Universitario de Málaga Avenida Carlos Haya s/n, 6Malaga, Spain, 7Novartis Pharma AG, Basel, Switzerland, 8Hospital Universitario de Burgos Avda. Islas Baleares s/n, Burgos, Spain

Background: Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by isolated thrombocytopenia, with platelet counts <100x10^9/L. Eltrombopag is an oral small-molecule nonpeptide thrombopoietin-receptor agonist that has shown to increase platelet counts. The safety and tolerability of this management of patients with chronic ITP (aged ≥1 year) who are refractory to other treatments (eg, corticosteroids, immunoglobulins). The recommended eltrombopag dose in patients with chronic ITP is 25mg once daily, OD (East Asians, 12.5mg OD or 25mg every other day) in adults and pediatrics aged 6-17 years at initiation, followed by dose adjustment to a maximum of 75mg OD based on platelet counts. REVIEU study was conducted in accordance with risk management plan in five European Union (EU) countries to document eltrombopag utilization patterns in real-world practice. Here, we report the eltrombopag utilization data on the subset of adult patients (aged ≥18 years) with ITP as primary diagnosis.

Aims: To evaluate the real-world data to determine drug utilization patterns among adult patients with ITP receiving eltrombopag within five EU countries.

Methods: REVIEU study was a multinational, multicenter, retrospective, medical chart review in patients with a documented past treatment with eltrombopag between the period immediately after first approval/launch in May 2010 and September 2014 (ie, dispensed at least once by the pharmacy and patient received at least one dose) for whatever reason. Patients who participated or were participating in a randomized eltrombopag clinical trial were excluded.

Table 1

<table>
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<tr>
<th>Table 1. Proportion of patients with platelet counts by ITP disease phase, dose, and by eltrombopag.</th>
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<tbody>
<tr>
<td>ITP disease phase</td>
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<td>Chronic</td>
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<td>Acute</td>
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<td>Chronic</td>
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Results: Overall, 287 adult patients with ITP (chronic [≥12 months], 73.5%; persistent [≥3-12 months], 10.8%; acute [≥3 months], 13.6%; unknown [n=1]) were included, majority in Spain (n=128) followed by Italy (n=67), Greece (n=36), France (n=29), and Germany (n=27). Eltrombopag was the first treatment with no prior ITP therapies in 12 (4.2%) [acute, 10.3%; persistent, 6.5%; chronic, 2.8%] patients. A total of 99 (34.6%) patients received one prior therapy (corticosteroids, 79 [27.6%], 128 (44.8%) patients received two prior therapies (corticosteroids+immunoglobulins, 114 [39.9%], 47 (16.4%) patients received three prior therapies (corticosteroids, immunoglobulins, and splenectomy). In total, the majority of patients received at least one prescription of corticosteroids (252, 88.1%) followed by immunoglobulins (180, 62.9%), and splenectomy (64, 22.4%) prior to eltrombopag initiation. Patients received an average daily dose of eltrombopag 45.6mg (chronic ITP, 44.6mg; persistent ITP, 43.1mg; acute ITP, 53.0mg) during the study. Overall, dose changes were reported in 749 adult ITP prescriptions (down-titration, 53.7%; up-titration, 43.7%; no change in dose, 2.7%). 49.1% of dose changes were reported during the first 6 months of treatment (36% in first 3 months). The main reasons for dose change included: disease improvement (30.4%), no treatment response (26.8%) and others (27.1%). Disease improvement accounted for down-titration in 51.2% (206/402) and up-titration in 4.6% (15/327), and no treatment response for up-titration in 54.4% (178/327) and down-titration in 5.0% (20/402) of adult patients with ITP. The majority of patients with platelet counts by ITP disease phase, and by eltrombopag dose are reported in Table 1.

Summary/Conclusions: The majority of adult patients with ITP (75.3%) were diagnosed with chronic ITP, and were treated with eltrombopag as second-line or greater therapy after corticosteroids and immunoglobulins, in line with the approved indication. Eltrombopag was also prescribed in 24.4% of adult patients with acute and persistent ITP. The starting dose followed the summary of product characteristics (SmPC) recommendations in the majority of cases and dose modifications were generally according to platelet counts. Data from REVIEU study have shown that eltrombopag use in the real world setting is largely consistent with the EU label and is considered part of ITP medical therapies.
Summary/Conclusions: This study shows a significant higher platelet desialylation in ITP patients who are non-responders to conventional therapies, particularly if they are also refractory to TPO-RA. According to a previous study (1), these results seem to be associated to platelet activation mediated by anti-platelet specific antibodies.

Reference

P722

SEQUENTIAL USE OF THROMBOPOIETIN RECEPTOR AGONISTS IN ADULT PRIMARY IMMUNE THROMBOCYTOPENIA PATIENTS: A RETROSPECTIVE COLLABORATIVE SURVEY FROM ITALIAN HEMATOLOGY CENTERS


Background: ITP is a disorder characterized by thrombocytopenia resulting from both increased immune-mediated platelet clearance and inappropriate thrombopoiesis. TPO-RAs—romiplostim (R) and eltrombopag (E) - offer a new opportunity of treatment with high response rates. However, a small fraction of pts does not respond or loses response - i.e. desired platelet (plt) count achieved but not sustained over time - during long-term follow-up, which can not be resumed even if dosage is increased over time, or experience wide fluctuations in plt counts with either agent. Moreover, adverse events (AE) may necessitate treatment discontinuation. Finally, patient’s preference may be an important issue considering the different route and timing of administration of the two agents and the alimentary restrictions needed for proper E absorption.

Availability of two TPO-RAs for clinical use, with different molecular structure and site of binding within the TPO receptor, has prompted trials of TPO-RA switching with the aim of overcoming treatment limitations of either agent resulting in reported overall response rates of approximately 80% in poor responders to both increased immune-mediated platelet clearance and inappropriate thrombopoiesis.

Methods: Charts of ITP pts receiving TPO-RAs at 17 collaborating Haematology Centers. Results were reviewed: demographics, CDRs, AE, response, and outcome.

Results: A total of 291 pts were included in the study. 106 pts were receiving TPO-RA. Data were collected in a dedicated case report form. Pts were grouped and analyzed based on the clinical setting prompting the switch (Table 1). The study was approved by the Hospital Review Board of each participating Center.

Table 1.
Results: A total of 546 pts received either R or E between Dec 2009 and Dec 2015. Of these, 106 (19.4%) underwent TPO-RA switch. Table 1 summarizes outcome after switch. Overall 69/106 (65%) of pts achieved, regained or maintained response upon switching. Either one TPO-RA switch outcome was equally effective (p=0.882). Outcome was not associated with gender, age at 1st TPO-RA treatment, splenectomy status. However, number of lines of previous therapies was associated with lower response: n=10, p=0.020); each additional line of therapy yielded a 30% increase in the odds of being a non-responder; a trend toward lower probability of response was observed in pts with longer lasting disease before 1st TPO-RA administration (p=0.086). Adverse events (AE; 16/106 pts) were generally mild and reversible upon discontinuation of either one TPO-RA. 1 study with thrombocytenia (one patient) and severe (standard anticoagulation) thrombotic events were observed which did not recur after switching. AE were characteristic of older pts: each additional year increase in pts age determined a 5% increase in the odds of developing AE.

Summary/Conclusions: Approximately 20% of TPO-RA treated pts were felt by treating physicians to be potentially worthwhile candidates for switching therapy. Exposure to the 2nd TPO-RA was more effective in pts who had lost response to 1st TPO-RA (80% responders) compared to those who were non responders to 1st TPO-RA (49% responders, p=0.001). It could be speculated that lack of response to either one of the two available TPO-RA identifies a subgroup of pts less likely to respond to a second available TPO-RA. Pts switched for non-efficacy reasons are more likely to maintain a response upon switch (p=0.030). The so far unexplained and unprecedented phenomenon of wide platelet fluctuation appears to be linked to the removal of the spleen, the physiological platelet reservoir organ.

### Table 1.

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<thead>
<tr>
<th>Occurrence of events after TPO-RA treatment</th>
<th>Discontinued</th>
<th>Interrupted</th>
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<tr>
<td>Total</td>
<td>69/106</td>
<td>37/106</td>
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<tr>
<td>Discontinued due to AE</td>
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<td>Discontinued due to non-therapy</td>
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<td>Discontinued due to death</td>
<td>1/106</td>
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<td>Discontinued due to study end</td>
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PT23

THROMBOEMBOLIC EVENT MANAGEMENT AND OUTCOMES IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENA (cITP) DURING TREATMENT WITH ELTROMBOPAG (EPAG): RESULTS FROM THE EXTEND STUDY

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Background: EPAG is an oral thrombopoietin receptor agonist approved for treatment of previously treated patients (pts; eg corticosteroids, immunoglobulins) with cITP aged ≥1 yr. The EXTEND study, a global, open-label, extension study of pts with cITP who received EPAG or placebo in prior EPAG studies, evaluated long-term safety and tolerability of EPAG. In EXTEND, 19 (6.3%) pts receiving EPAG experienced a total of 24 thromboembolic events (TEEs; Table 1) from 30 Dec 2010–28 Feb 2016, which is similar to TEE incidence in cITP pts receiving romiplostim (Kuter et al. Br J Haematol 2013;161:411–23) and to one estimate in the general cITP population (Sarpaltwari et al. Haematologica 2010;95:1167-75).

Aims: To describe management and outcomes of TEEs occurring during EPAG treatment in the EXTEND study.

Methods: Adult pts with cITP received EPAG starting at 50mg/day, with titration to 25–75mg per day or less as required, based on individual platelet count. If the TEE was potentially caused by a switching event, exposure to the 2nd TPO-RA was more effective in pts who had lost response to 1st TPO-RA (80% responders) compared to those who were non-responders to 1st TPO-RA (49% responders, p=0.001). It could be speculated that lack of response to either one of the two available TPO-RA identifies a subgroup of pts less likely to respond to a second available TPO-RA. Pts switched for non-efficacy reasons are more likely to maintain a response upon switch (p=0.030). The so far unexplained and unprecedented phenomenon of wide platelet fluctuation appears to be linked to the removal of the spleen, the physiological platelet reservoir organ.

Results: 302 pts were enrolled and received ≥1 EPAG dose; 67% female; 38% <60 yrs old. Median exposure duration was 2.4 yrs (range, 2 days to 8.8 yrs) and mean daily dose was 50.2 (range, 1-138) mg. Summary/Conclusions: This analysis shows that most pts who experienced a TEE had resolution of the event after medical/surgical treatment, most commonly anticoagulant therapy, regardless of whether EPAG was discontinued, interrupted or continued. The decision to restart EPAG following a TEE should be made on a case-by-case basis, with caution (including frequent platelet count monitoring) and only if the benefit is expected to outweigh any risk. If anticoagulation therapy is instituted (as in most cases), it is possible the bleeding risk may shift the risk-benefit to maintenance of EPAG treatment.

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PT24

SEVERE BLEEDING IN THE ELDERLY WITH PRIMARY IMMUNE THROMBOCYTOPENIA: CHARACTERISTICS, RESPONSE TO THERAPY AND LONG-TERM OUTCOME

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1State Key Laboratory of Experimental Hematology, Institute Of Hematology And Blood Diseases Hospital, Chinese Academy Of Medical Sciences And Peking Union Medical College, Tianjin, 2Department of Hematology, Affiliated Suzhou Hospital of Nanjing Medical University (Suzhou Municipal Hospital), Suzhou, China

Background: Primary immune thrombocytopenia (ITP) is often diagnosed in the elderly. The elderly patients have been reported to have a higher incidence of severe bleeding manifestations and a higher ITP-related mortality. Nonetheless, few data exist on the characteristics and long-term prognosis of elderly patients with severe bleeding. Aims: We retrospectively evaluated elderly patients with ITP who had severe bleeding to determine characteristics, response to therapy and long-term outcome.

Methods: We reviewed the medical records of 517 ITP patients over 60 years of age diagnosed at our center (192 men and 325 women) between 1991 and 2012. Therapy was started at diagnosis or during follow up. Bleeding severity was assessed by the Mazzucconi’s bleeding assessment. Logistic regression analysis was used to determine which presenting features were associated with the risk of severe bleeding. Cox regression analysis was used to estimate rate ratios (RR) for no remission and mortality.

Results: Among 517 patients with ITP, 10 (1.9%) presented intracerebral hemorrhage (ICH) and 74 (14.3%) presented severe (non-ICH) bleeding during ITP. According to multivariable analysis, risk of severe bleeding in patients was increased with platelet count <10×10^9/L (P=0.001, OR=1.682, 95% CI 1.271–2.234), female patients (P=0.010, OR=2.148, 95% CI 1.200-3.844), complication (P=0.038, OR=3.049, 95% CI 1.292-7.232), which is similar to TEE incidence in cITP patients who received romiplostim (Kuter et al. Br J Haematol 2013;161:411–23) and to one estimate in the general cITP population (Sarpaltwari et al. Haematologica 2010;95:1167-75).

Summary/Conclusions: This analysis shows that most pts who experienced a TEE had resolution of the event after medical/surgical treatment, most commonly anticoagulant therapy, regardless of whether EPAG was discontinued, interrupted or continued. The decision to restart EPAG following a TEE should be made on a case-by-case basis, with caution (including frequent platelet count monitoring) and only if the benefit is expected to outweigh any risk. If anticoagulation therapy is instituted (as in most cases), it is possible the bleeding risk may shift the risk-benefit to maintenance of EPAG treatment.
patients with severe bleeding. At the end of follow-up, the estimated 10-year cumulative rate of no remission among patients with severe bleeding was higher than that among patients without severe bleeding (P=0.017, RR=1.608, 95% CI, 1.052-2.456). The estimated 10-year cause-specific mortality related to fatal bleeding in patients with severe bleeding was higher than that in patients without severe bleeding (P=0.001, RR=9.886, 95% CI, 1.806-54.098). The estimated 10-year mortality among ICH patients was higher than that among severe (non-ICH) patients (P=0.009, RR=5.543, 95% CI, 1.317-15.688).

Summary/Conclusions: Platelet count <10×10^9/L, female patients, complication of pulmonary disease, gum or oral mucosal bleeding and epistaxis are significant predictive factors for severe bleeding in the elderly. Severe bleeding in elderly ITP was associated with more failure of response to treatment, increased long-term risk of no remission and mortality related to fatal bleeding.

P725
ATORVASTATIN IMPROVE THE PROGNOSIS OF ADULT PATIENTS WITH CORTICOSTEROID-RESISTANT IMMUNE THROMBOCYTOPENIA VIA ENHANCING BONE MARROW ENDOTHELIAL CELL FUNCTION

Y. Kong1, X.-N. Cao1, X.-H. Zhang1, M.-M. Shi1, Y.-Y. Lai1, Y.-Q. Sun1, Y. Wang1, L.-P. Xu1, Y.-J. Chang1, X.-J. Huang1
1Peking University People’s Hospital, Peking University Institute of Hematology, Peking University, Beijing, China

Background: Immune thrombocytopenia (ITP) is generally considered to be an autoimmune disorder characterized by increased peripheral platelet destruction and reduced platelet production. Corticosteroids represent the standard first-line therapy achieving responses in around 80% of patients. However, for those corticosteroid-resistant ITP patients, who exhibit either no response (NR) to corticosteroids or corticosteroid-dependent, the pathogenesis remains poorly understood and the management is challenging. Emerging evidence from mouse studies has suggested that the cross-talk between megakaryocytes (MKs) and bone marrow progenitor cells (EPCs) in the bone marrow (BM) microenvironment regulates MKs maturation and thrombopoiesis. We recently reported that the impaired BM EPCs, which could be quantitatively and functionally improved by atorvastatin in vitro, induced the occurrence of poor graft function following allo-transplantation (Blood, 2016, 128:2988-2999). However, little is known about the functional role of BM EPCs and how to improve impaired BM EPCs in patients with corticosteroid-resistant ITP.

Aims: To determine whether quantitative and/or functional abnormalities of BM EPCs are involved in the occurrence of corticosteroid-resistant ITP. Moreover, to investigate the effects of atorvastatin and N-Acetyl-L-cysteine (NAC, a ROS scavenger) on the number and function of cultured BM EPCs derived from patients with corticosteroid-resistant ITP and its underlying molecular mechanisms.

Methods: Twenty-three patients with corticosteroid-resistant ITP, 30 patients with newly diagnosed ITP and 17 healthy donors (age 18-55) were enrolled from 2016 to 2017 at Peking University Institute of Hematology. BM EPCs were cultured as previously reported. Atorvastatin and NAC were administrated to the 5-day cultured BM EPCs in corticosteroid-resistant ITP patients until tested on day 7. The number and function of BM EPCs were evaluated pre- and post-treatment by DiI-Ac-LDL and FITC-lectin. The BM EPCs were then stained for CD34, VEGFR2, and CD133 and then analyzed by flow cytometry and western blot. Subsequently, a single-center pilot study was performed to evaluate the efficacy and safety of atorvastatin and NAC to adult patients with corticosteroid-resistant ITP.

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Results: Human bone marrow EPCs were demonstrated as the spindle shape and the similar expression of CD34, VEGFR2 and CD133 at day 7 of cultivation among all patients. Compared three cohorts of subjects and dysfunctional BM EPCs, which were characterized by impaired proliferation, migration, angiogenesis, and higher levels of ROS and apoptosis, were revealed in corticosteroid-resistant ITP patients compared to those in newly diagnosed ITP. Activation of p-P38 was detected in BM EPCs from corticosteroid-resistant ITP patients. Furthermore, the number and function of BM EPCs derived from corticosteroid-resistant ITP patients were enhanced by atorvastatin or NAC treatment in vitro through down-regulation of the p38 mitogen-activated protein kinase (MAPK) pathway. In the single-center pilot study, a total of 12 corticosteroid-resistant ITP patients were recruited to receive either the combination of atorvastatin and NAC or alone. At day 14 post-randomization, the percentages of platelets positive for Rucinus communis agglutinin I (RCA-I), Erythrina cristagalli lectin (ECL) or Succinyl Triticum vulgare lectin (sWGA) analyzed by flow cytometry represented the levels of platelet desialylation. Platelet response was defined as platelet counts ≥50×10^9/L were randomly assigned to receive antimicrobial therapy alone (control group) or antimicrobial therapy plus oseltamivir (oseltamivir group). The study flowchart is shown in Fig. 1. Both groups received appropriate antimicrobial agents and standard medical support based on the guidelines issued by the Surviving Sepsis Campaign. The oseltamivir group additionally received 5 full days of oseltamivir therapy. The oseltamivir was administered orally or through a feeding tube at a dose of 75mg once every 12 hours. Time from randomization to the administration of oseltamivir was less than 24 hours. The antimicrobial agents were continuously administered until 3 days after the resolution of the physiological abnormalities related to the systemic inflammatory response syndrome (SIRS). The primary outcomes were platelet desialylation level at study entry, and overall platelet response rate within 14 days post-randomization. Secondary outcomes included platelet recovery time, the occurrence of bleeding events, and the amount of platelets transfused within 14 days post-randomization. The percentages of platelets positive for Rucinus communis agglutinin I (RCA-I), Erythrina cristagalli lectin (ECL) or Succinyl Triticum vulgare lectin (sWGA) analyzed by flow cytometry represented the levels of platelet desialylation. Platelet response was defined as platelet counts returning to or above 100×10^9/L. Platelet recovery time was calculated as the date of recovery to the date when platelet counts were >100×10^9/L. Written informed consents were obtained from the study participants prior to inclusion in the study.

Figure 1.
Results: The platelet desialylation levels increased significantly in the 127 septic patients with thrombocytopenia compared to the 134 patients without thrombocytopenia. A platelet response was achieved in 45 of the 54 patients in the oseltamivir group (83.3%) compared with 34 of the 52 patients in the control group (65.4%; P=0.045). The median platelet recovery time was 5 days (interquartile range 4–6) in the oseltamivir group compared with 7 days (interquartile range 5–10) in the control group (P=0.003). The amount of platelets transfused decreased significantly in the oseltamivir group compared to the control group (P=0.044). The multivariate analysis by Cox proportional hazards models showed that the Sequential Organ Failure Assessment (SOFA) score and platelet recovery time were independent indicators of oseltamivir therapy.

Summary/Conclusions: Thrombocytopenia was associated with increased platelet desialylation in septic patients. The addition of oseltamivir could significantly increase the platelet response rate, shorten platelet recovery time and reduce platelet transfusion. Chinese Clinical Trial Registry, ChiCTR-IPR-160008542.

PT27
SAFETY AND EFFICACY OF LONG-TERM OPEN-LABEL DOSING OF SUBCUTANEOUS (SC) ROMIPLOSTIM IN CHILDREN WITH IMMUNE THROMBOCYTOPENIA (ITP)
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Background: Children with ITP for ≥6 months who completed a romiplostim phase 1/2 or phase 3 parent study could enroll in this open label long term extension study

Aims: To evaluate the safety and efficacy of long-term romiplostim in children with ITP.

Methods: Patients enrolled at 28 sites in the US, Canada, Spain, and Australia. All patients received SC romiplostim once weekly. The initial dose was the final dose from the parent study or 1 µg/kg for patients previously receiving placebo; dose was then adjusted from 1-10 µg/kg to target platelet counts of 50–200×10^9/L. Incidence of adverse events (AEs) was the primary endpoint.

Results: As of 24 Feb 2016, 66 patients entered this study; 65 received romiplostim for up to 6.2 years. At baseline, median (min–max) age was 11 (3–18) years; 56% were female; 61% were white, 14% African American, 14% Hispanic/Latino, 9% Asian, and 3% other; 9.1% had prior splenectomy. Median (min–max) baseline platelet count was 27.5 (2–458)×10^9/L. Median (min–max) treatment duration was 100 (5–321) weeks. Median (min–max) average weekly romiplostim dose was 4.8 (0.1–10.0) µg/kg, which included escalation to a stable dose. After ~week 200 (n =8 patients), the median dose was observed to fluctuate. All 65 patients received their doses per protocol >90% of the time; 18 patients missed ≥1 dose due to noncompliance for a total of 41 times. Reasons for discontinuing treatment (n=22, 33%) included consent withdrawn (n=8), required other therapy (n=4), noncompliance (n=3), administrative decision (n=3), per protocol (n=1), and AE (n=2) (asthenia, headache, dehydration, and vomiting in one patient and anxiety in the other, per investigator, none of the AEs were treatment-related); 43 (65%) patients continued in the study. Fifty-two serious AEs occurred in 17 patients, 3 deemed treatment-related (anemia, epistaxis, and thrombocytopenia). Bleeding AEs occurred in 56 patients; 5 deemed treatment-related (gingival bleeding, petechiae, injection site bruising, injection site hematoma, and epistaxis). No thrombotic events were reported. There were no peripheral blood abnormalities warranting a bone marrow examination. No patients had anti-TPO neutralizing antibodies. From week 2 on, median platelet counts remained >50×10^9/L; platelet counts were >100×10^9/L at most timepoints, despite an observed decrease in the median dose from 4-5 µg/kg to 2-3 µg/kg around week 160 (Figure). Nearly all (94%, 61/65) patients had a platelet response (median platelet counts for a month ≥50×10^9/L). Nine (14%) patients (5 boys and 4 girls, none with prior splenectomy) entered remission (Table), defined here as platelet counts ≥50×10^9/L for 24 weeks with no ITP treatments. Twenty-three (35%) patients received rescue medications.

Summary/Conclusions: Over 6 years of data from this ongoing open-label extension study of romiplostim in children with ITP show that >90% of children achieved a platelet response with romiplostim. The safety profile was overall tolerable, similar to that in past studies. Some children (9/66) with longstanding ITP entered remission after receiving romiplostim.
Quality of life, palliative care, ethics and health economics 2

P728
IMPACT OF VENEToclAX ON THE QUALITY OF LIFE OF CLL PATIENTS RELAPSED/REFRACTORY TO B-CELL RECEPTOR (BCR) SIGNALING PATHWAY INHIBITOR TREATMENT

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Background: The prognosis for patients with CLL after B-Cell Receptor inhibitor (BCRi) failure is very poor. Patients with R/R CLL who discontinue and/or progress on BCRi treatment tend to have poor clinical outcomes. Venetoclax (VEN) is an oral BCL-2 inhibitor that has demonstrated deep and durable responses in relapsed/refractory (R/R) CLL patients.

Aims: To assess whether VEN has an impact on health related quality of life (HRQoL) among CLL patients R/R to BCRi treatment and receiving VEN monotherapy.

Methods: The study enrolled patients with CLL who had previously received treatment with ibritumomab and/or idelalisib, have relapsed on treatment, or experienced progression after discontinuation of either agent. Patients are to receive VEN monotherapy for up to two years, or until discontinuation due to disease progression, unacceptable toxicity, or any other reason. Patient-reported HRQoL measures included the EORTC QLQ-C30 and EORTC QLQ-CLL16, which were assessed at Baseline (BL), Week 24, and every 12 weeks thereafter. Mean change from BL to each assessment through Week 48 are reported here. Clinical relevance was based on minimum important difference (MID) of values from BL to each assessment. A change of 5-10 points is considered a “small” change on the EORTC-QLQ-C30. The lower bound of 5 points was used for MID acceptance on both measures.

Results: Decreases in all HRQoL domains from BL were observed early and were sustained throughout the entire follow-up period. Fatigue and role functioning demonstrated the most clinically meaningful improvements across all time points. The summary HRQoL score (T1-48) assessed as a whole showed a mean improvement of 5,4 and MID was reached at the first assessment (5,4; p=021).

Summary/Conclusions: This interim analysis provides preliminary evidence that demonstrates CLL patients R/R to BCR inhibitors receiving VEN monotherapy experienced improvement in several key aspects of functioning and HRQoL. These results may be important to consider when making therapeutic choices in R/R CLL following relapse or progression on BCRi inhibitors.

P729
THE ROLE OF PSYCHOLOGICAL VARIABLES FOR TYROSINE KINASE INHIBITORS (TKI) DISCONTINUATION IN CHRONIC MYELOID LEUKAEMIA (CML) PATIENTS: IMPLICATION FOR MEDICAL DECISION MAKING PRACTICE

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Background: Treatment-free remission (TFR) is an emerging goal for CML patients (pts) that reach a sustained deep molecular response (DMR), as it can reduce the risk of long-term toxicities that impair quality of life, and mitigate the costs associated with long-term TKI therapy. Therapy discontinuation may represent a great challenge for patients and different factors (not only clinical) may play a role in medical decision, such as psychological and emotional variables.

In this respect, it is essential to consider pts’ concerns and preferences regarding the discontinuation option.

Aims: The study was aimed at investigating psychological (emotional and cognitive) and clinical factors related with the attitude to opt for discontinuation of therapy in CML pts.

Methods: This is an observational, prospective, no-drug related study conducted in 3 Italian centers with large experience in CML treatment. A detailed battery of questionnaires focusing on health behaviour, risk taking and personality was administered.

Results: One hundred and twenty pts were enrolled (56% males; mean age=50, SD=1.2). Median duration of the disease was 8 years (range 1-39y). 62/120 pts were receiving Imatinib first line. The idea of stopping TKI was appealing in 71% of pts. In 81% of pts there was a high probability of responders upon restarting a TKI. Pts are more likely to keep their TKI if the risk of relapse is no more than 30% (% Mean=33.62; SD=33.46). Main worries related with the choice to stop TKI are fear of possible disease recurrence, (60.5%), fear of drug resistance if the disease relapses (44.5%) and fear to disappoint family or friends (26.8%). Older pts (>40 years) are more concerned about relapse and subsequent lack of response than younger (x2=9.65, p=0.02). Finally, pts with higher passive risk taking attitude (who are more reluctant and undecided in everyday-life decisions) seemed to be more afraid to lose disease control in CML. ANOVA showed a significant difference between two groups (F=5.54; p=0.021).

Summary/Conclusions: Many studies have confirmed the feasibility and safety of stopping TKI therapy in selected pts, with the potential to drastically modify clinical practice in CML management in the next future. TKI discontinuation appears appealing and challenging at the same time for many CML pts. This study, for the first time, analyses how and when pts would consider this option including implications for health care providers in clinical practice, using both a clinical and psycho-cognitive perspective.

P730
BUDGET IMPACT ANALYSIS OF BIOSIMILAR RITUXIMAB (CT-P10) FOR THE TREATMENT OF CHRONIC LYMPHOCYTIC LEUKAEMIA IN THE 28 EU MEMBER STATES

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Background: In December 2016, the European Medicines Agency’s Committee for Medicinal Products for Human Use has recommended granting marketing authorisation to biosimilar rituximab (CT-P10) in all indications of the reference product, including chronic lymphocytic leukaemia (CLL). Compared to the originator rituximab, significant price reductions are expected offering a more affordable treatment option for CLL patients across Europe.

Aims: To assess the budget impact of the introduction of CT-P10 into the treatment of CLL in the 28 EU member states. Moreover, we provide an estimation of the number of additional CLL patients that can be treated with CT-P10 from the cost savings.

Methods: A budget impact analysis was performed to evaluate the one-year cost outcomes under two scenarios with and without the availability of CT-P10. The budget impact was calculated as the difference in costs between the two scenarios. For the major European markets, five-year cost savings were also estimated. Market uptake of CT-P10 was assumed to be 30%. A third party payer’s perspective was adopted, and only drug costs were considered. Based on expert opinion, it was assumed that when CT-P10 is entering the market it will be at 50-70% of the official list price of originator rituximab in each country. Costs of administration and monitoring were not incorporated in the calculations, as it can be assumed that these are equal for the reference product and CT-P10. The initial number of patients treated with rituximab was estimated from IMS sales data on total annual consumption of originator rituximab in 2016. Other model parameters such as patients’ average body surface area and treatment rate of rituximab among CLL patients, were derived from the published literature. One-way sensitivity analysis was undertaken to test the robustness of model assumptions.

Results: Over a one year time horizon, the cumulative budget impact of adopting CT-P10 is estimated to be €17.80 million in the 28 EU member states (30% discount in drug prices compared to the originator rituximab). Countries responsible for the majority of the cost savings are Germany (€4.06 million), Italy (€3.15 million), France (€2.41 million), Spain (€1.50 million), the UK (€1.34 million), Poland (€0.80 million), Austria (€0.66 million), the Netherlands (€0.59 million), Finland (€0.49 million) and Sweden (€0.43 million). If the cost savings were used to treat additional CLL patients with CT-P10, a total of 6,124 patients could be treated annually throughout Europe. The potential cost savings are in a direct correlation with the price and market uptake of CT-P10. Applying a 40% and 50% discount in drug prices compared to the originator rituximab, cost savings are projected to €23.73 and €29.67 million, from which further
2,526 and 2,706 CLL patients could be treated with CT-P10, respectively. Over a 5-year period, the total cost savings are expected to reach €29.81 million in Germany, €23.10 million in Italy, €17.65 million in France, €10.98 million in Spain and €9.83 million in the UK.

Summary/Conclusions: Biosimilar rituximab has the potential to improve the affordability of CLL treatments and ease the burden of healthcare costs in Europe. The results of this study could be used to inform the development of a positive reimbursement strategy for CT-P10. Using the cost savings to treat additional patients would substantially increase the access to better cancer medications, and thus contribute to a longer survival as well as better quality of life outcomes in CLL.

P731
AN INVESTIGATION INTO THE NEEDS AND PREFERENCES OF PATIENTS WITH MULTIPLE MYELOMA DURING REMISSION–IMPLICATIONS FOR RE-DESIGNING PATIENT-CENTRED HEALTHCARE SYSTEMS

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Background: Therapeutic advances in multiple myeloma (MM) mean that patients have extended periods of remission without need for active anti-myeloma therapy. This provides an opportunity to review how these patients are managed and design patient-centred healthcare systems. Remote monitoring systems have been implemented for other cancer patients in remission.

Aims: We aimed to explore patient needs during stable remission from MM and evaluate the acceptability of remote monitoring.

Methods: Patients with stable MM in a treatment-free interval selected from outpatient clinics at a tertiary centre completed a survey which explored the acceptability of various methods of remote monitoring. Subsequently semi-structured interviews were conducted by an independent researcher to investigate factors influencing this preference. Interviews were conducted until saturation of themes, transcribed verbatim and thematic analysis was performed using open coding by a doctor, psychotherapist and psychologist.

Results: 78 patients were surveyed; the most acceptable alternative was a telephone clinic (with doctor 77%, nurse 69%). 19 interviews were conducted exploring suitability of telemedicine for MM. Patients preferred TC as an alternative to FTF consultations replacing clinical face to face (FTF) consultations with a doctor. Median age was 61 years (range 46-76), and 9 were male. 18 patients were in 1st remission; 16 had most recently received high dose therapy and autograft, 3 had post autograft consolidation. The centre was not the local hospital for 18 patients interviewed. The majority were accepted to TC as an alternative to FTF clinics due to the burden of travel, associated cost and clinic waiting times. These affected patients’ physical and psychological well-being, with TC perceived as less burdening. Patients acknowledged reduced needs during remission compared to treatment phase and felt TC would benefit redistribution of consultant time for patients on active therapy. Some suggested this service change would be beneficial for healthcare resourcing rather than them personally. Interpretation of blood results by clinicians was regarded as central to monitoring disease, and for some who were unaware of clinical symptoms, the only way a relapse would be detected. General preference was for bloods to be done locally, leading to concerns about availability of results for TC. Patients were unsure how to monitor their own MM, hence valued the knowledge of their medical team. Doctors were perceived to have more expertise than nurses and this influenced preferences regarding who undertook TC. As a result, patients sought reassurance they could see a doctor if they had any concerns after TC with a nurse. Patients valued ongoing contact under the centre where they were treated due to prior positive experience and the importance of being seen at a tertiary centre renowned for its expertise in MM. This influenced acceptability of TC as long as they remained under the centre’s care with preference for continuity of staff involved. Whilst TC was acceptable for patients in remission, some were concerned about how relapse would be managed and expressed preference for FTF when being told they had relapsed.

Summary/Conclusions: Nurse led TCs are an acceptable alternative to FTF consultations for monitoring patients in remission from MM. Design of healthcare systems incorporating TCs need to have robust systems for accessing blood test results, for managing relapse, ready access to doctors and reassurance about the competence and knowledge of practitioners involved.

P732
COST-EFFECTIVENESS OF RITUXIMAB IN ADDITION TO STANDARD OF CARE CHEMOTHERAPY FOR ADULT PATIENTS WITH ACUTE LYMPHOCYTIC LEUKAEMIA

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Background: In The Group for Research on Adult Acute Lymphoblastic Leukemia (GRAALL-R), the addition of monoclonal antibody rituximab to standard chemotherapy for Philadelphia chromosome-negative, CD20-positive, B-cell precursor Acute Lymphoblastic Leukemia (CD20+ Ph- BCP-ALL) resulted in improved clinical outcomes. However, the cost-effectiveness of rituximab for this indication has not been previously evaluated. We attempted to examine this question in the context of the Canadian publicly funded health care system.

Aims: To determine the economic impact in Canada of the addition of rituximab to standard of care (SOC) chemotherapy vs SOC alone in newly diagnosed CD20+ Ph- BCP-ALL.

Methods: Standard of care consisted of the two most widely used chemotheraphy regimens for adults with ALL in Canada: hyper-CVAD or the Dana Farber Cancer Institute (DFCI) ALL consortium. A decision analytic model included the following health states over a 15-year time-horizon: event-free survival, relapsed/resistant disease, cure (death from causes other than ALL, given ≥5 years event free survival), overall survival, and death. Event specific acceptability (SAE) rates were calculated. Costs of the model included: first-, second- and third-line treatment and administration; disease management; palliative care; and SAE-related treatments. Model inputs were sourced from public data, literature and provincial cancer agency inputs. Results are presented using probabilistic sensitivity analysis and Monte Carlo simulation incorporating uncertainty around all model inputs.

Results: Life years increased by 1.33 years (95%CI: 0.10-2.63 years) with rituximab in addition to SOC vs SOC alone. Quality-adjusted life-years (QALYs) increased by 1.15 QALYs (95%CI: 0.34-1.93 QALYs) with rituximab in addition to SOC. The incremental cost of rituximab plus SOC was C$46,624 (95%CI: C$28,881-$56,515), chiefly due to the drug acquisition costs of rituximab. Superior relative EFS associated with rituximab in addition to SOC drove lower second-line treatment and palliative care use, resulting in modest cost savings. The resulting mean Incremental Cost-Effectiveness Ratio (ICER) was C$40,505/QALY. At a willingness-to-pay threshold of C$100,000/QALY, the probability of being cost-effective was 96%. Decision outcomes were robust to the probabilistic and deterministic sensitivity analyses, including the SOC backbone as either hyper-CVAD or DFCI.

Summary/Conclusions: For adults with CD20+ Ph- BCP-ALL, rituximab in addition to SOC is a cost-effective intervention compared to SOC alone, from a Canadian public payer perspective. Rituximab is associated with increased survival as well as better quality of life outcomes in CLL. However, the cost-effectiveness of rituximab for this indication has not been previously evaluated. We attempted to examine this question in the context of the Canadian publicly funded health care system.

P733
THE THERAPEUTIC UTILITY OF A SYSTEMATIC PROTOCOL FOR GERIATRIC ASSESSMENT IN ONCOHEMATOLOGICAL PATIENTS

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Background: The prevalence of hematological malignancies has increased over time especially in the older population. In the era of immunochemotherapy and target therapy, it is important to have a multidimensional and comprehen- sive approach that allows the optimization of standard of care by using either intensive therapeutic measures or a more conservative approach. To choose the best treatment for these patients, a geriatric hematology program has been launched.

Aims: Evaluate the utility of the comprehensive geriatric assessment (CGA) in patients with hematologic malignancies on the initial therapeutic decision making. Determine frailty prevalence and short- mid term prognostic impact using a screening tool for its identification.

Methods: Patients diagnosed with hematologic malignancies were followed prospectively. Patients age 70 and over were referred to hematologusal nursing consultation. 88 screening tool was used to identify frailly risk. Patients with frailty criteria and those requiring geriatric evaluation were referred for a comprehensive geriatric consultation at the Internal Medicine and the Oncology Clinic for carrying out the CGA. In the comprehensive geriatric assessment, the clinical information obtained included: physical, mental, social and nutritional assessments, as well as an additional screening on geriatric syndromes. The regular medication was reviewed based on the STOPP/START criteria. The patients were classified in 3 categories according to the Balducci classification: 1) Fit, 2) Fragile and 3) Poor prognostic.

Results: We have included 32 patients in the last 9 months, with an average age of 81 (71-89) years. 56% of the sample was female. The main hematologic malignancy referred was high grade non-Hodgkin lymphoma (59%). At the time of the evaluation, 87% had ECOG 1 and 30% had CR at the last evaluation. The social, functional and mental profiles are shown in Table 1. According to polypharmacy and comorbidities, data are shown in Table 2. The distribution of patients by frailty scales, are described in Table 3. 56% of the patients were classified as robust, 35% fragile and the rest with poorly prognosis. After the evaluation we recommended nutritional measures, control of the polypharmacy and physical exercise. Of the included patients, 22 had been reviewed at 6 months staying alive 95%. 24% required hospitalization after the initial assessment and 13% went to the emergency department.
for birth weight (large for gestational age) to control confounding. Cases with Down syndrome were excluded from the analyses.

Results: Overall, 13 cases (1.2%) and nine controls (0.3%) had a record indicating at least one CT examination. Of the relevant CT scans, 50% were performed on the head region and 41.3% the thorax region. The median age at CT scan was 8.12 years (7.46 years for cases and 10.0 years for controls). In a conditional logistic regression analysis adjusted for birth weight, a significantly increased leukemia risk (OR=4.75, 95% CI 1.55, 14.5) was found for any CT examination (one or more) at least two years prior to leukemia diagnosis. When comparing one CT examination and two or more CT examinations with no examinations the ORs were respectively 2.78 (95% CI 0.73, 10.5) and 16.9 (95% CI 1.81, 150).

Summary/Conclusions: In our preliminary analyses we observed a substantial increase in childhood leukemia risk related to pediatric CT scans. The risk estimates are materially higher than in two earlier studies\(^1,2\) and need to be interpreted with caution. We will seek to estimate radiation doses to the red bone marrow, based on limited data available on CT examinations (body part and examination type).

References

P734
RADIATION EXPOSURE FROM CT IMAGING AND CHILDHOOD LEUKEMIA: A NATIONWIDE CASE-CONTROL STUDY
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Background: Pediatric CT imaging offers significant benefits in clinical practice. However, children are more sensitive to carcinogenic effects of ionizing radiation than adults and red bone marrow is especially radiosensitive tissue type. The risk estimates of low doses of ionizing radiation are mainly\(^1,2\) based on extrapolated results of studies done with substantially higher radiation doses and there exists a need to assess the risks of low doses with a more direct approach.

Aims: We assessed the leukemia risk in children after computed tomography imaging studies with high-quality Finnish register data and data from hospital databases.

Results: Down syndrome were excluded from the analyses. Conditional logistic regression analyses were adjusted for birth weight (large for gestational age) to control confounding. Cases with Down syndrome were excluded from the analyses.

Results: Overall, 13 cases (1.2%) and nine controls (0.3%) had a record indicating at least one CT examination. Of the relevant CT scans, 50% were performed on the head region and 41.3% the thorax region. The median age at CT scan was 8.12 years (7.46 years for cases and 10.0 years for controls). In a conditional logistic regression analysis adjusted for birth weight, a significantly increased leukemia risk (OR=4.75, 95% CI 1.55, 14.5) was found for any CT examination (one or more) at least two years prior to leukemia diagnosis. When comparing one CT examination and two or more CT examinations with no examinations the ORs were respectively 2.78 (95% CI 0.73, 10.5) and 16.9 (95% CI 1.81, 150).

Summary/Conclusions: In our preliminary analyses we observed a substantial increase in childhood leukemia risk related to pediatric CT scans. The risk estimates are materially higher than in two earlier studies\(^1,2\) and need to be interpreted with caution. We will seek to estimate radiation doses to the red bone marrow, based on limited data available on CT examinations (body part and examination type).

References

P735
HEALTHCARE RESOURCE UTILIZATION WITH IXAZOMIB OR PLACEBO PLUS LENALDIDIODE-Dexamethasone in the Randomized, Double-Blind, Phase 3 Tourmaline-MM1 Study in Relapsed/Refractory Multiple Myeloma (RMM)
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Background: Treatment paradigms for RMM have evolved in recent years with the approvals of multiple novel agents and evidence of benefits for using triplet vs doublet therapy and continuous treatment until progression. With more complex regimens and longer treatment duration, costs of treatment and healthcare resource utilization (HRU) are expected to increase, with IV agents having a greater impact on treatment burden than oral agents. The oral proteasome inhibitor ixazomib is approved in the US, EU, and multiple countries worldwide, in combination with lenalidomide-dexamethasone (Rd), for the treatment of RMM patients (pts) following at least 1 prior therapy. Approval was based on the phase 3 TOURMALINE-MM1 study of ixazomib-Rd vs placebo-Rd, which demonstrated significantly improved progression-free survival (PFS; median 20.6 vs 14.7 months, HR 0.74) with ixazomib-Rd, with limited additional toxicity and no adverse impact on patient-reported quality of life (QoL; Moreau et al, N Engl J Med 2016).

Aims: HRU was an exploratory endpoint of the TOURMALINE-MM1 trial. The aim of this analysis was to compare HRU with ixazomib-Rd vs placebo-Rd, incorporating all non-protocol additional medical care encounters such as inpatient and outpatient admissions and their duration, as well as time lost from work or other activities by pts and their caregivers.

Methods: 722 RMM pts with 1-3 prior lines of therapy received ixazomib 4mg (n=360) or matching placebo (n=362) on days 1, 8, and 15, plus lenalidomide 25mg on days 1-21 and dexamethasone 40mg on days 1, 8, 15, and 22, in 28-day cycles until disease progression or unacceptable toxicity. The primary end-point was PFS. HRU was assessed on day 1 of each cycle prior to treatment and every 4/12 weeks during PFS/overall survival follow-up. After a median follow-up of 23 months, pts had received a median of 17 (range 1-34) and 15 (1-34) cycles of ixazomib-Rd and placebo-Rd, respectively; HRU data are reported from this analysis time point.

Table 1.
Results: Overall, 152 (42%) pts on the ixazomib-Rd arm had 316 hospitalization events, compared to 156 (43%) pts (335 events) on the placebo-Rd arm. Exposure-adjusted hospitalization rates (0.530 and 0.564 per pt-year [ppy], respectively) and mean length of stay (10 and 10.8 days) were similar between the ixazomib-Rd and placebo-Rd arms (Table 1). Rates of outpatient visits were also similar between arms; 217 (60%) pts on the ixazomib-Rd arm had 197 (median 4) compared to 198 (55%) pts and 194 vs 199 visits (median 5) on the placebo-Rd arm. Exposure-adjusted visit out rates were 3,305 and 3,355 ppy, respectively (Table 1). On the ixazomib-Rd arm, 46 (13%) pts missed a total of 527 (median 7 days) of work or other activity, compared to 51 (14%) pts and 580 (median 8) days on the placebo-Rd arm. Similarly, 16 (4%) pts caregivers missed 1428 (median 5) days of work or other activity on the ixazomib-Rd arm, compared to 24 (7%) pts caregivers and 110 (median 4) days on the placebo-Rd arm.

Summary/Conclusions: The ixazomib-Rd triplet regimen did not add to the HRU burden compared to the placebo-Rd doublet, while prolonging PFS. Treatment was consistent with the limited additional toxicity burden and the reported lack of an adverse impact on QoL with ixazomib-Rd. In contrast to findings reported for injected agents (Armoiry et al, J Clin Pharm Ther 2011; Gaultney et al, J Clin Pharm Ther 2013; Baz et al, Support Care Cancer 2015), this all-oral triplet regimen did not increase time lost from work, caregiver burden, or the number of inpatient/outpatient visits.

P737

EFFECT OF IMPROVEMENT OF SURVIVAL, POPULATION AGING AND IMWG 14 CRITERIA ON INCIDENCE AND PREVALENCE OF MULTIPLE MYELOMA

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Background: There are some variables that can modify Multiple Myeloma incidence of New Diagnosed (NDMM) and prevalence over the time: Past decade shows a new demographic data in our society: the increment of expectancy of life and an excellent performance status. In the last years we have assisted to an amazing improvement in the management and expectancy of life of Multiple Myeloma (MM) patients. Recent changes in criteria recommendation by IMWG '14 to begin treatment in NDMM patients can increment its incidence. New expensive but very effective and well tolerated antimielyoma (antiMM) agents are in the center of attention of Hematologic and Public Healthcare Systems. There are data of improvement of survival that can increment of prevalence.

Aims: We have analysed our data base and calculate incidence by sex, age and three 5-years periods of time at diagnosis and obtain tendencies to get ready for next decade of ageing people with best antimielyoma agents. We have analysed prevalence of MM patients on last 7 years with cutoff date on 1st of November (2010 to 2016).

Methods: We retrospectively analysed the incidence of patients with new diagnostic of Multiple Myeloma (NDMM) from 1998 to 2012. (Fig.1). Then we divide the cohort in several groups: sex and age at diagnosis (3 groups: <65, 66-75 and >75) and in four 5-year (quinquennium) period of time (1998-2002, 2003-07, 2008-12, 2013-NOV2016). (Fig. 2). We have calculated the incidence per 100000 inhabit/year using census data of our Local Registry of Tumours of our Public Health Area. Characteristics of patients: n= 346. M/F: 206/140. Median age diagnosis: 74 years (Range: 39-100).

Results: A) INCIDENT RATES (see Table). In the past IMW (Roma-14#PO197) we reported incidence rates form 1998 to 2012. We observed a constant increase of Annual Average of incidence from 4.57 cases/100000 inhabitants/ year from the 1st period to 6.15 in the last. Adjusted by Age Incidence increase from 14 to 18.5 cases in the 065 group. From 2013 to Nov-2016 global and adjusted by age incidence remains similar to last years data with 80 new cases in the 4-year-period (5,9 cases for global population and 17,2 cases for over65 population). After IMWG ‘14 criteria to begin treat in NDMM the incidence was similar to the last 7 years (2008-12 period) incidence with 37 NDMM cases (25 O65y group).

B) PREVALENCE RATES (PreVR).

• 2012. 77 pts alive. PreVR: 22.2 /100000 inhabit.
• 2014. 84 pts alive. PreVR: 24.4/100000 inhabit.
• 2016. 103 pts alive. PreVR: 30.3 / 100000 inhabit.

Table 1.

Summary/Conclusions: Although we don’t observe substantial changes on incidence rates of NDMM, we have noted an important rise on prevalence rates of more than 40% from 2010 to 2016 (21.2 from 30.3 pats alive /100000 inhabit.) Several new antiMM drugs are available in the therapeutic arsenal and probably increases the prevalence rates.
HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ISOLATED EXTRAMEDULLARY RELAPSE OF ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDREN

BACKGROUND: Although most children affected by Acute Lymphoblastic Leukemia (ALL) are cured with current protocols, relapses still occur in the bone marrow as well in extramedulillary sites, mainly the central nervous system (CNS) and the skin.

METHODS: From 1990 to 2015, 281 children (1-18 years) underwent HSCT for ALL iEMR from 19 centers, members of the Italian Pediatric Onco-Hematology Association (AIEOP).

RESULTS: Of the 281 patients (203 male, 78 female) 167 presented relapse confined to CNS, 73 to tests, 14 to mediastinum, 11 to CNS + other sites and 18 to other organs. Thirty one percent of children experienced a late relapse, 34.5% an early relapse, 31% a very early relapse, for 3.5% the time of relapse is not known. Ninety-seven patients underwent auto HSCT, 79 MFD HSCT, 75 MUD HSCT and 30 Haplo HSCT. At transplantation 72.6% of children were in CR2, 21.0% in CR>2 and 6.4% were not in remission Total body irradiation (TBI) and conditioning regimens were applied. The overall survival for the entire cohort was 56% at 10 years and was not influenced by sex, lineage, age, site of relapse, length of first remission, HSCT type (Auto vs MFD vs MUD vs Haplo). Patients transplanted in CR2 had the better OS (64%), those in CR>2 the worse OS (44%), and those transplanted with disease in CR+2, even patients transplanted with disease were included in the analysis. If a matched familiar (MFD) or a matched unrelated donor (MUD) was available, HSCT was performed from one of these; if not, the single center decided to perform autologous HSCT (Auto HSCT) or haploidentical HSCT (Haplo HSCT). The only factors influencing outcome were number of CR and year of transplantation. Patients in CR>2 have a risk of death 2.3 times greater than those in CR2. Children treated after 2000 have half the risk of death than those treated from 1995 to 2000.

Summary/Conclusions: In this study we present the largest series of patients with ALL iEMR treated with HSCT with a very long follow up. Comparison with published data shows that the current approach is favorable, especially for early and very early relapse: in fact the use of HSCT seems to abrogate the impact of some “classical” negative risk factors. Our results suggest that both autologous and allogeneic HSCT are efficient treatments for ALL iEMR. Data from contemporaneous protocols will further clarify the role of HSCT in the treatment of ALL iEMR. Informed consent was obtained from parents or legal guardians. Treatment protocols were based on Berlin-Frankfurt-Münster (BFM) trials at our institution.

P740

DEFIBRITIDE EFFICACY AND SAFETY IN PATIENTS WITH HEPATIC VENO-OCLUSIVE DISEASE/SINUSOIDAL OBSTRUCTION SYNDROME (VOD/SOS) DIAGNOSED AFTER DAY 21: ANALYSIS OF FINAL DATA FROM AN EXPANDED-ACCESS PROGRAM

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Background: Hepatic VOD/SOS is a potentially life-threatening complication of conditioning for hematopoietic stem cell transplantation (HSCT). VOD/SOS has multi-organ dysfunction (MOD) may be associated with >80% mortality. Although VOD/SOS typically begins <21 days post-HSCT (per Baltimore/mod- ified Seattle criteria), late-onset VOD/SOS has been reported in 15%-20% of significant risk factors for developing VOD. In efforts to predict the risk for VOD in a patient who has received prior IO, we performed a classification and regression tree analysis (CART) and noted that the combination of VOD and a double alloantigen preparative regimen was significantly associated with the risk for developing VOD (HR 5.9, 95% CI 1.3-7.2, p=0.01) and receiving a busulfan-based transplant preparative regimen (HR 3.4, 95% CI 1.02-12, p=0.05); not receiving a prior SCT was significantly protective (HR 0.3, 95% CI 0.1-0.8, p=0.02). Number of IO cycles, time from IO to SCT, age, and donor relation were not found to be significant factors for developing VOD. In efforts to predict the risk for VOD in a patient who has received prior IO, we performed a classification and regression tree analysis (CART) and noted that the combination of VOD and a double alloantigen preparative regimen was significantly associated with the risk for developing VOD (HR 5.9, 95% CI 1.3-7.2, p=0.01).

Summary/Conclusions: Fatal VOD is a rare occurrence. However, IO exposure prior to SCT increases the risk for any VOD. Furthermore, IO exposure followed by a double alloantigen preparative regimen increases this risk nearly 6-fold, and should be avoided in these patients.
≥2mg/dL, painful hepatomegaly, weight gain >5%, or ascites—plus mandatory hematologic/laboratory evidence of VOD/SOS. Defibrotide (DF) is approved to treat severe hepatic VOD/SOS post-HSCT in the EU, and for hepatic VOD/SOS with renal or pulmonary dysfunction post-HSCT in the US.

Aims: This is an analysis of DF efficacy and safety in patients (pts) with late-onset VOD/SOS using final data from an expanded-access study.

Methods: The original expanded-access protocol required VOD/SOS per Baltimore criteria or biopsy by Day+35 post-HSCT, with MOD (renal/pulmonary) by Day+45. The study was amended to include pts with later-onset VOD/SOS, with or without MOD; VOD/SOS per modified Seattle criteria; and VOD/SOS after chemotherapy alone. Pts provided informed consent and received DF 25mg/kg/d (8.25mg/kg q6h) for a recommended 321 days. "Late-onset" in this post-hoc analysis was defined as diagnosis >21 days post-HSC, hematomyeloid/ultrasound data (EBMT criteria) were not available.

Results: Of 1000 HSCT pts with a confirmed diagnosis of VOD/SOS and receiving ≥1 dose of DF, 264 (26.4%) had late-onset VOD/SOS, of whom 139 (52.7%) had MOD. By 8/8 well-matched group, 95/264 (36.0%) were pediatric (aged ≤18 years; 51/65 [53.7%] with MOD) and 169/264 (64.0%) were adults (aged ≥16 years; 88/169 [52.1%] with MOD). Kaplan-Meier estimated survival at Day +100 (Figure) was 52.8% (95% CI, 46.5–58.7%) across all HSCT pts and 43.9% (95% CI, 35.4–52.0%) for pts with MOD; for pediatric pts, this was 60.4% (95% CI, 49.9–68.7%) overall and 45.4% (95% CI, 31.0–58.6%) for pts with MOD; for adults, Day +100 survival was 48.7% (95% CI, 40.9–56.0%) overall and 43.0% (95% CI, 32.5–53.0%) for pts with MOD. Adverse events (AEs) occurred in 75.4% of the total group (80.6% with MOD); 70.5% of pediatric pts (76.0% with MOD); 78.1% of adults (83.0% with MOD). Treatment-related AEs (TRAES) occurred in 20.8% overall (23.7% in those with MOD); 21.1% of pediatric pts (23.5% with MOD); 20.7% of adults (23.9% with MOD). The most common TRAEs (>3%) were epistaxis, pulmonary hemorrhage, gastrointestinal hemorrhage, and hematuria (each in <5%) of pts. TRAEs leading to study discontinuation (n=25) or death (n=10), the most common survival pulmonary hemorrhage.

Support: Jazz Pharmaceuticals.

Background: Allogeneic stem cell transplantation (SCT) using HLA-matched unrelated donor (URD) has been usually regarded as a subsequent option in patients with severe aplastic anemia (SAA), who have failed to immunosuppressive treatment (IST). However, recent improved outcomes of URD SCT lead to its extended role for treating those lacking HLA-matched sibling donor (MSD).

Aims: Through this study, we intended to verify the possibility of URD SCT as a front-line treatment for SAA patients.

Methods: We compared outcomes of consecutive SAA patients who received SCT from 8/8 well-matched URD (WM-URD; n=61) and partially (6/8 or 7/8) matched URD (PM-URD; n=33) with 8/8 matched MSD (n=126) at our institution between Mar 2002 and Dec 2016. Patients receiving MSD and URD SCT were conditioned with fludarabine (180mg/m²) + cyclophosphamide (100mg/kg IV) plus rabbit ATG (10mg/kg IV), and total body irradiation (fractionated 800cGy) + cyclophosphamide (100-120mg/kg IV) with/without rabbit ATG (2.5mg/kg IV).

Results: Median age of the WM-URD and the PM-URD groups were significantly lower compared to that of the MSD group (29 yrs, 31 yrs, and 39 yrs; P <0.01), with a high proportion of those experiencing IST failures before SCT (80.3%, 90.9%, and 33.3%; P<0.01). Median days to neutrophil engraftment of the MS group was significantly shorter compared to those of the WM-URD and PM-URD groups (11 days, 16 days, and 16 days, respectively). The incidences of acute and chronic GVHD of the WM-URD and PM-URD groups were significantly higher compared to those of the MSD group (42.6% and 63.6% vs 9.5%; P<0.01, and 44.8% and 33.3% vs 8.9%; P<0.01, respectively). When we compared the incidence of transplant-related mortality (TRM): 10.7% vs 7.4%; P=0.53 and overall survival rate (OS): 89.3% vs 92.5% at 6 yrs; P=0.52) between the WM-URD and the MS group, there were no significant difference. However, trends of higher TRM incidence (18.2% vs 7.4% at 6 yrs; P=0.05) and lower OS rate (81.8% vs 92.5% at 6 yrs; P=0.05) were observed between the PM-URD and the MS groups. There was no primary graft failure (0%) and severe graft failure (0%) in both the URD group (0% vs 18.3%; P<0.01) and PM-URD (0% vs 18.3%; P=0.02) groups were significantly lower compared that of the MS group. When we adjusted other clinical and transplant-related factors, which include age and IST failure, using multivariate
analysis, the OS rate of the WM-URD group was not significantly different (HR 1.45, 95% CI; 0.52-4.09; P=0.48), whereas that of the PM-URD group was significantly lower (HR 2.85, 95% CI; 1.01-8.02; P=0.04), compared to that of the MSD group.

**Summary/Conclusions:** Our study showed that there was no significant difference in OS rate between the WM-URD and the MSD groups. As high incidence of GVHD remains a problem in the former group, strategies to reduce it are needed in future protocols.

**P743**

**HAPLOIDENTICAL ALLOGENEIC STEM CELL TRANSPLANT IN SEVERE THALASSEMIA PATIENTS**

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**Background:** Thalassemia free survival after allogeneic stem cell transplantation (SCT) is about 80–90% with either matched related or unrelated donors. However, the probability of finding a HLA-compatible donor is less than 50%. We explored the use of a mismatched related (“Haplo-”) donor.

**Aims:** To evaluate the outcome of SCT with Haplo donors in severe thalassemia patients.

**Methods:** All patients received two courses of pre-transplant immunosuppression therapy (PTIS) with fludarabine (Flu) 40mg/m2/2 together with dexamethasone (Dxm) 25mg/m2 for 5 d to facilitate engraftment. After two courses of PTIS, a reduced-toxicity conditioning regimen of rabbit anti-thymocyte globulin (ATG) 1mg/kg every day on days SCT-12,-11,-10, Flu 35mg/kg on days SCT -7,-6,-5,-4,-3,-2 and IV Busulfan (Bu) 130mg/m2 on days SCT -7,-6,-5,-4 was given followed by T-cell replete peripheral blood progenitor cells (PBPC). GVHD prophylaxis consisted of cyclophosphamide (Cy) 50mg/kg on days SCT -3 and -4 (Post-Cy), and on day SCT +5 tacrolimus or sirolimus was started together with a short course of mycophenolate mofetil.

**Results:** Fifty-one patients underwent haplo-SCT. Their median age was ten years (range, 2 to 28 years). Forty-nine patients engrafted with 100% donor chimerism. Two of five patients with high titers of donor-specific anti-HLA antibodies suffered primary graft failure. Median time to neutrophil engraftment was 14 days (range, 11 to 18 days). Eight patients developed mild to moderate, reversible veno-occlusive disease, while twelve patients developed acute GVHD grade II, that quickly responded to steroid therapy. Only seven patients developed limited chronic GVHD. Projected overall and event-free survival rates at two years are 95% and 94%, respectively. The median follow up time is 18 months (range;10 to 50 months).

**Summary/Conclusions:** This haplo-SCT protocol may yield excellent outcomes for thalassemia patients, and provide a treatment option for patients lacking a HLA-matched donor.

**P744**

**AUGMENTATION OF FLUDARABINE AND BUSULFAN-BASED MYELOABLATIVE REGIMEN WITH THIOTEPA IMPROVES OUTCOMES WITH NO ADDED TOXICITY IN ALLOGENEIC STEM CELL TRANSPLANTATION FOR ACUTE MYELOID LEUKAEMIA**

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**Background:** Allogeneic stem-cell transplantation (HSCT, allo-SCT) is the most effective way to control leukemia relapse for patients with acute myeloid leukemia (AML). Busulfan and Cyclophosphamide (Bu/Cy), the current standard of care, in allogeneic transplant for acute myeloid leukemia (AML), is limited by the increased treatment related mortality. Myeloablative doses of Busulfan (12-8mg/kg) with Fludarabine (160mg/m2) (Flu-Bu), has reduced toxicity, however with the limitation of increased relapses. We have tried to improve outcome of Flu-Bu regimen by augmentation with Thiopeta (10mg/kg). Here we compared outcomes of 45 such patients (getting augmented regimen, Flu-Bu with the addition of Thiopeta, (group 2), to 44 patients who received Fludarabine, Busulfan myeloablative reduced toxicity regimen (group 1), during the same period.

**Aims:** The primary objective of the report was to compare the toxicity and incidence of relapse between the two regimens. Secondary objective was to compare overall survival (OS), and disease-free survival (DFS), the non-relapse mortality (NRM), engraftment kinetics, incidence of acute and chronic graft versus host disease (GVHD), and comparison between high and low-risk patients amongst the two groups.

**Methods:** 89 patients with AML were retrospectively analyzed. 44 patients were conditioned with Flu-Bu (group 1) and 45 patients augmented with Thiopeta (Flu-Bu-TT, group 2). The transplant conditioning regimen, (augmented myeloablative) consisted of 30mg/m2 intravenous Fludarabine for 5 days (total dose 150mg/m2), for matched related donors or for 6 days (180mg/m2), for unrelated or mismatched donors, intravenous Busulfan (3.2mg/kg/day for 4 days, total dose 12.8mg/kg), and intravenous Thiopeta 5mg/kg for 2 days (10mg/kg). The conventional myeloablative regime was identical, however without the addition of Thiopeta.

**Results:** Toxicities were comparable, with mucositis in 7 patients (15%) in group 1 and 8 patients (17%) in group 2, (p=1.0), severe sepsis in 4 (9%) in group 1 and 3 (6%) in group 2, (p=0.7), severe venocclusive disease in 2% in group 1 and 4% of group 2, (p=1.0) and comparable non- relapse mortality (NRM) in group 1 and 2, (p=1.0). 5-year disease free survival (DFS) median follow up of 5 years, was significantly better in group 2, 38% for group 1, and 62% in group 2, (p=0.02) and 5-year overall survival showed trend towards benefit in group 2 (62% vs 42%, p=0.06). 14/30 (46%) patients in group 1 relapsed, as compared to 4/31 patients, (12%, p=0.005) in group 2, considering NRM as competing risk.

**Summary/Conclusions:** In conclusion, the outcome of augmented regimen (DFS and OS) is superior Flu-Bu regime, mainly due to reduction in relapses, with comparable toxicities and could eventually replace Bu/Cy.
in intervals of 5% +/- 2% (e.g. 65 +/-2% probability of survival at 1 year). Corresponding observed 1 year OS was then estimated for each group by the KM method. A kernel smoother was used to visually display the average of observed 1 year survival estimates over the continuous range of predicted OS. Results: 506 patients with AMI (n=290), ALL (n=72), or MDS (n=144) were included. Of these, 470 patients (AMI=263, MDS=141, ALL=66) had full data available for the CIBMTR Calculator. On univariate and multivariate analyses, DRI, HCT-CI, and age correlated with significant differences in OS/RFS, while donor HLA match correlated with a significant difference in OS. Stratifying patients based on a composite of DRI (low/intermediate vs high/very high) and HCT-CI (0-2 vs 3+) revealed significant differences in OS/RFS between the 4 groups (Fig. 1). Compared with a reference group of patients with both low/intermediate DRI and low HCT-CI, those with high DRI and low HCT-CI were at greater risk of death (HR 2.30; 95% CI 1.39-3.81) and relapse or death (HR 2.50; 95% CI 1.55-4.05), more so than patients with a higher HCT-CI but still low/intermediate DRI (HR death 1.80; 95% CI 1.34-2.43; HR relapse/death 1.68; 95% CI 1.26-2.24). When comparing predicted and observed survival, KM estimates of 1 year OS fell within range of that predicted by the CIBMTR Calculator in almost all groups (Fig. 1). In one group, patients had lower observed 1 year OS than predicted (76%, 95% CI 62-93%, vs 85 +/- 2%, p=NS). In this group, 29/30 patients (97%) had intermediate or high DRI; 59% had poor prognostic ALL by NCCN criteria (n=12, 44%) or other adverse features such as minimal residual disease pre-HCT (n=4, 15%).

Figure 1.

Summary/Conclusions: Based on a large cohort of patients who underwent CD34 alloHCT for acute leukemia or MDS, we demonstrate that DRI is a major determinant of outcome. The CIBMTR Survival Outcomes Calculator predicts 1 year prognosis with relative precision, though some disease-risk features not reflected in the Calculator may affect outcomes in patients with otherwise good prognosis. Taken together, these prognostic models can assist in predicting outcomes and identifying patients most likely to benefit from CD34 allo-HCT. Furthermore, applying the CIBMTR calculator analysis in individual centers may help identify patients with worse outcomes than predicted and guide patient and/or HCT selection.

P746

THROMBOTIC MICROANGIOPATHY AFTER ALLOGENEIC STEM CELL TRANSPLANTATION: IS THERE A PROTECTIVE ROLE FOR URSODEOXYCHOLIC ACID?

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Background: Thrombotic microangiopathy (TMA) after allogeneic stem cell transplantation (alloSCT) may be a severe complication associated with high mortality. Since there is no standard treatment it would be helpful to have efficacious prophylactic measures. Some data support the beneficial effect of ursodeoxycholic acid (UDA) to prevent endothelial-cell damage. We retrospectively analysed a total of 616 patients transplanted (1995-2016) requiring systemic treatment (the original one) to those with just severe cGVHD systemic treatment. In 2016 EMBT annual meeting a redefinition of this endpoint was proposed changing cGVHD event from those patients with cGVHD requiring systemic treatment (as the original one) and we had compared both.

Aims: We retrospectively analysed a total of 671 patients undergoing to prophylaxis with sirolimus-tacrolimus (SRL/TKR), prior transplant and non-UDA patients. The probability of overall TMA at 180 days in UDA patients was 9.6% (95% CI: 5.9-14.3), versus 14.7% (95% CI: 11.7-18.1) in non-UDA patients. On multivariate analysis the risk factors which remained statistically significant were unadjusted donor and (in the case of SRL/TKR, whereas the use of UDA significantly decreased the risk of TMA (HR:0.4, 95% CI:0.2-0.8, p=0.01). Moreover, in the subgroup of SRL/TKR, 100 days-cumulative incidence of TMA was 11.8% (95% CI: 6.9-18.1) versus 25.6% (95% CI: 17.9-33.9) depending on the use or not of UDA, respectively (p:0.005), whereas in the subgroup of CNL/MTX 100d-Cumulative incidence of TMA was 3.4% (95% CI: 0.6-10.6) vs 12.1% (95% CI: 7.1-18.6) with and without UDA, respectively (p:0.05).

Summary/Conclusions: In conclusion the use of UDA decreases the risk of TMA after alloSCT regardless of type of immunophrophylaxis.

Table 1.

| Aims: We had generated two composite endpoints: in both III-IV aGVHD, relapse or death were considered events but we defined GFRS1 as the one with cGVHD event including those who required systemic treatment (as the original one) and in GFRS2 just those with severe cGVHD (the EBMTC redefined one) and we had compared both.

Methods: We retrospectively analysed 616 patients transplanted (1995-2016) excluding non-malignant diseases, second allo-SCT and those <16 years old age.

P747

FACTORS PREDICTING GRAFT VERSUS HOST DISEASE-FREE, RELAPSE-FREE SURVIVAL AFTER ALLOGENEIC TRANSPLANTATION. COMPARISON ATTENDING TO TWO DIFFERENT DEFINITIONS AND BENEFIT OF HAPLOIDENTICAL DONOR
Results: Characteristics of patients are shown in Table 1. With a median follow-up for patients alive of 39 months (3-221), the median estimated survival in months and the % at +1 year and +2 years was: 114 months, 70% and 62% overall survival (OS); 23 months, 57% and 49% event free survival (EFS); 6 months, 35% and 26% GRFS1; 11 months, 46% and 38% GRFS2. 147 (24%) and 218 (35%) hadn’t any event in GRFS1 and in GRFS2 respectively. In GRFS1, event incidence was: 90 (15%) for III-IV aGVHD, 170 (27%) for cGVHD, 152 (25%) for relapse and 75 (9%) for death; In GRFS2 was 90 (15%), 65 (11%), 174 (28%) and 65 (11%) respectively. Considering those patients with cGVHD as event in GRFS1, 105 of them hadn’t the event as cGVHD at the same time in GRFS2 (since they had cGVHD requiring systemic treatment but not severe cGVHD). For these patients, the alternative event in GRFS2 was: 72 without any event, 22 relapsed and 11 died. In the multivariate analysis, factors associated with better outcomes were: for GRFS1 diagnosis (p=0.04; benefit in NHL/HL/CLL p=0.02, HR 0.71; CI95% 0.53-0.95), >4 prior lines (p=0.03, HR 1.5, CI95% 1.04-2.04), early EBMT stage (p<0.001 with early as reference; intermediate p=0.002, HR 1.5, CI95% 1.2-1.9; advance p=0.001, 2.0, 1.5-2.6), in vivo T-cell depletion (p=0.02, 0.6, 0.39-0.92) and haploidentical donor (p=0.04 with HLA identical as reference, no significance 1 or 2 mismatch [p=0.18], haploidentical p=0.02, 0.43, 0.25-0.74). Only early EBMT disease stage maintained significance in GRFS2 (p=0.001 with early as reference; intermediate p=0.005, 1.5, 1.1-1.9; advance p=0.001, 1.9, 1.4-2.6).

Summary/Conclusions: In our study the percentage of the GRFS endpoint was similar to previously reported. Comparing both proposed definitions, the GRFS2 endpoint define a higher population of patients without any event; so that it is possible that morbidity is misdiagnosed. The EBMT disease score was the factor with more impact in both; it is interesting to point that although the group, haploidentical donor is associated with better GRFS1.

P748

EFFICACY AND SAFETY OF DEFIBROTIDE IN THE TREATMENT OF HEPATIC VENO-OCLUSIVE DISEASE/SINUSOIDAL OBSTRUCTION SYNDROME FOLLOWING HEMATOPOIETIC STEM CELL TRANSPLANTATION: FINAL SUBGROUP RESULTS

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Background: Hepatic veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS) is a potentially life-threatening complication of conditioning regimens for hematopoietic stem cell transplant (HSCT) and may also occur following chemotherapy without HSCT. VOD/SOS with multi-organ dysfunction (MOD) may be associated with >80% mortality. Diagnosis has traditionally been based on the Baltimore criteria or modified Seattle criteria. Defibrotide is approved for treating severe hepatic VOD/SOS post-HSCT in the European Union and for treatment of hepatic VOD/SOS with renal/pulmonary dysfunction post-HSCT in the United States. The defibrotide expanded-access protocol was designed to provide access to defibrotide prior to its approval in the United States and to collect additional data on safety and efficacy in a broader patient population, including those with and without MOD, and following HSCT or chemotherapy without HSCT.

Aims: This is an analysis of defibrotide efficacy and safety in the subgroup of patients developing VOD/SOS following HSCT, using final data from the expanded-access protocol.

Methods: The original expanded-access protocol required VOD/SOS diagnosis by either criteria or biopsy post-HSCT, with evidence of MOD (27%) or pulmonary dysfunction). The study was amended to also include patients without MOD (off-label), with VOD/SOS per modified Seattle criteria, and/or with VOD/SOS following chemotherapy without HSCT (off-label). After patients provided informed consent, defibrotide treatment (25mg/kg/d in 4 divided doses of 6.25mg/kg) was recommended ≥21 days.

Results: This analysis of final data is based on 1000 patients enrolled from 2007–2016 who had confirmed VOD/SOS following HSCT and had received ≥1 dose of defibrotide. Of these patients, 512 (51.2%) had MOD. The median age was 14 years (range 0.10–77.0), with 570 patients (57.0%) aged ≤16 years, (28% by age 14 years, 23% only of whom had MOD) and 430 patients (43.0%) aged >16 (231 (45.1%) of whom had MOD). Among pediatric patients, 28.2% were aged <1–23 months, 52.5% aged 2–11 years, and 19.3% aged 12–16 years. Primary diseases in ≥10% of the overall HSCT group were acute lymphocytic leukemia (19.8%), acute myelogenous leukemia (26.1%), and neuroblastoma (10.5%). Kaplan-Meier estimated Day+100 survival was 58.8% (95% confidence interval [C.I] 55.7%–61.9%) in the overall HSCT group (Figure), with rates of 49.5% (95% CI, 45.0%–53.8%) in patients with MOD and 68.9% (95% CI, 64.5%–72.9%) in patients without MOD. In patients aged ≥16 years, Kaplan-Meier estimated Day+100 survival was 67.9% (95% CI, 63.8%–71.6%) and 47.1% (95% CI, 42.3%–51.8%) in patients aged >16 years (Figure). In the overall HSCT population, 120 patients (21.0%) had ≥1 treatment-related adverse event (TRAEE). TAREs occurring in ≥2% of patients were pulmonary hemorrhage (4.6%), gastrointestinal hemorrhage (3.0%), epistaxis (2.3%), and hypotension (2.0%).

Support: Jazz Pharmaceuticals.
P749

GENERATION OF IMMORTAL MURINE HEMATOPOIETIC STEM/PROGENITOR CELL LINES FROM TRANSGENIC MICE

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Background: Research on hematopoietic and leukemic stem cells (LSCs) is currently limited as these cells are infrequent and their immortalization is hardly achievable.

Aims: We aimed to establish a long term ex-vivo culture system that allows maintenance and expansion of LSC (lin-, Sca-1+, c-Kit+) cells.

Methods: We adapted a technique described by the L. Carlsson lab and transduced high-purity sorted murine LSKs with Lhx2, a LIN-homeobox transcription factor, which has been reported to facilitate ex vivo expansion of immature hematopoietic cells.

Results: Lhx2 expressing-hematopoietic progenitor cell (HPC(LSK)) lines require SCF (stem cell factor) and IL-6 and they can be maintained in a feeder-independent culture for more than 6 months. They preserve LSK markers despite continuous proliferation. HPC(LSK)-cells repopulate lethally irradiated mice and re-feed the murine hematopoietic cell pool. HPC(LSK)-cells were established from a range of transgenic mice, underlying the overall applicability of this model. Using this system, we established LSC lines that express BCR/ABLp210, MLL-AF9,NrasG12V or Flt3-ITD; NrasG12D. These LSCs home to the bone marrow, differentiate into all lineages and drive myeloid leukemia in mice.

Summary: We created the most efficient method of expanding hematopoietic stem/progenitor cells. They are immortalized and can be expanded indefinitely. This tool allows analysis of the molecular mechanisms controlling self-renewal in hematopoietic and LSCs as well as drug screening. Our system may represent a breakthrough in (cancer) stem cell biology and assist in the development of new therapeutic avenues to combat LSCs.

OUTCOMES.

P750

INHIBITING BCL2 AND NK CELLS IMPROVES STEM CELL TRANSPLANT OUTCOMES.

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Background: Allogeneic haematopoietic stem cell transplantation (alloHSCT) is the most effective means of preventing relapse of blood cancers, in particular AML. The curative potential of alloHSCT is largely due to the immune mediated graft-versus-leukemia (GVL) effect, which in turn is dependent on the stable engraftment of donor immunity. The dual challenge of alloHSCT is therefore being able to protect the donor HSC and allow sufficient donor engraftment for haematopoietic and immunological reconstitution that drives the GVL effect while limiting the toxicity of conditioning and the onset of graft-versus-host disease (GVHD).

Aims: Optimize the use of BCL2 inhibitors to modify recipient NK cell function in models of alloHSCT in order to minimize GVHD severity and onset.

Hypothesis: Therapeutic targeting of recipient NK cell frequency or function pre-transplant will allow reduced intensity conditioning (RIC) and promote both donor T cell engraftment and GVHD whilst reducing the risks of GVHD.

Methods: We used a MHC-mismatched mouse model of alloHSCT, where donor and T cells from BALB/c (H2Kd) mice were injected into irradiated C57BL/6 (H2Kb) recipients. On day 0, C57BL/6 WT, or Bcl2−/− mice were treated on day -2 and -1 by oral gavage with 2 x 600 rad, or 2 x 400 rad, before iv injection of 7.5e6 BALB/c BM + 1e6T cells. C57BL/6 WT mice were also treated on day -2 and -1 by oral gavage in NKp46+NK1.1+cells) recipients were irradiated with either a standard dose of 2,5Gy whereas non-irradiated MSC from the same sample were used as controls. MSC were characterized following ISCT criteria (flow cytometry and in vitro differentiation stainings). Apoptosis was evaluated by flow cytometry using annexinV/7AAD staining. Expression microarrays of irradiated and control-MSC were performed using Human Gene 2.0 ST Array platform (Affymetrix). RT-PCR of key genes involved in the hematopoietic supporting capacity as well as in the differentiation of MSC into osteoblasts and adipocytes was performed in both experimental groups. Finally, long term BM cultures (LT-BMC) were performed as functional assays to test the hematopoietic-supporting ability of irradiated and non-irradiated MSC. For the analysis of robust survival endpoints, CD34+ cells were isolated from leukaemias and seeded on stromal layers from non-irradiated or irradiated MSC. CFU-GM colonies derived from the LT-BMC were scored weekly.

Results: Flow cytometric characterization of irradiated MSC was comparable to that of control MSC. Similarly, there were no differences in the percentage of viable cells between both experimental groups neither at one hour nor at 72h post irradiation, confirming once more the radio-resistance of MSC. In addition, expression arrays did not show any statistically significant differences in genes involved in hematopoiesis maintenance. However, upon comparing the differentiation ability we interestingly observed that irradiated-MSC differentiation was skewed towards osteogenesis whereas adipogenesis was impaired. In this regard, irradiated-MSC had significantly higher SPP1 expression (involved in late osteogenic differentiation) and lower CBPA and PPAR-gamma (both genes involved in adipogenesis) compared to control MSC. After inducing in vitro differentiation, there were no differences in ALP and Alizarin Red staining but the number of adipocytes per field at days 7, 14 and 21 was significantly lower in irradiated MSC (p=0,018 p=0,046 and p=0,018). In addition, angioptin and SDF-1, key genes implicated in maintenance of hematopoiesis, were significantly overexpressed in irradiated-MSC (p=0,043 and p=0,028, respectively). Finally, in the functional evaluation of the hematopoietic-supporting ability of MSC by LT-BMC, we observed that the number of CFU-GM colonies generated by the culture was significantly higher in the irradiated group after 4 and 5 weeks (p=0,046 and p=0,018, respectively) compared to the non-irradiated group. Furthermore, the number of adipocytes per field was significantly reduced in the LT-BMC. In summary, irradiation of MSC with 2,5Gy improves their hematopoietic supporting ability and modifies their differentiation capacity, increasing the osteogenesis and decreasing the adipogenesis.

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P752

DYSFUNCTION OF BONE MARROW MESENCHYMAL STEM CELLS FROM PATIENTS WITH PROLONGED ISOLATED THROMBOCYTOPENIA CAN BE IMPROVED BY N-ACETYL-L-CYSTEINE

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Background: Mesenchymal stromal cells (MSC) are precursors of adipocytes and osteoblasts in the bone marrow (BM) niche, and key regulators of the hematopoietic process. After HSC transplantation, MSC remain of host-origin. Total body irradiation has been widely used in conditioning regimen and MSC are shown to be radio-resistant. Nevertheless, the functional effects of irradiation on BM-MSC have not been extensively explored.

Aims: The main objective was to evaluate the effects of irradiation on the MSC in their hematopoietic-supporting capacity.

Methods: Ten BM samples were obtained from healthy donors after informed consent. MSC were obtained and characterized following standard procedures previously described. Then, one aliquot was gamma-irradiated with a single dose of 2,5Gy whereas non-irradiated MSC from the same sample were used as controls. MSC were characterized following ISCT criteria (flow cytometry and in vitro differentiation stainings). Apoptosis was evaluated by flow cytometry using annexinV/7AAD staining. Expression microarrays of irradiated and control-MSC were performed using Human Gene 2.0 ST Array platform (Affymetrix). RT-PCR of key genes involved in the hematopoietic supporting capacity as well as in the differentiation of MSC into osteoblasts and adipocytes was performed in both experimental groups. Finally, long term BM cultures (LT-BMC) were performed as functional assays to test the hematopoietic-supporting ability of irradiated and non-irradiated MSC. For the analysis of robust survival endpoints, CD34+ cells were isolated from leukaemias and seeded on stromal layers from non-irradiated or irradiated MSC. CFU-GM colonies derived from the LT-BMC were scored weekly.

Results: Flow cytometric characterization of irradiated MSC was comparable to that of control MSC. Similarly, there were no differences in the percentage of viable cells between both experimental groups neither at one hour nor at 72h post irradiation, confirming once more the radio-resistance of MSC. In addition, expression arrays did not show any statistically significant differences in genes involved in hematopoiesis maintenance. However, upon comparing the differentiation ability we interestingly observed that irradiated-MSC differentiation was skewed towards osteogenesis whereas adipogenesis was impaired. In this regard, irradiated-MSC had significantly higher SPP1 expression (involved in late osteogenic differentiation) and lower CBPA and PPAR-gamma (both genes involved in adipogenesis) compared to control MSC. After inducing in vitro differentiation, there were no differences in ALP and Alizarin Red staining but the number of adipocytes per field at days 7, 14 and 21 was significantly lower in irradiated MSC (p=0,018 p=0,046 and p=0,018). In addition, angioptin and SDF-1, key genes implicated in maintenance of hematopoiesis, were significantly overexpressed in irradiated-MSC (p=0,043 and p=0,028, respectively). Finally, in the functional evaluation of the hematopoietic-supporting ability of MSC by LT-BMC, we observed that the number of CFU-GM colonies generated by the culture was significantly higher in the irradiated group after 4 and 5 weeks (p=0,046 and p=0,018, respectively) compared to the non-irradiated group. Furthermore, the number of adipocytes per field was significantly reduced in the LT-BMC. In summary, irradiation of MSC with 2,5Gy improves their hematopoietic supporting ability and modifies their differentiation capacity, increasing the osteogenesis and decreasing the adipogenesis.
**Background:** Prolonged isolated thrombocytopenia (PT), is a serious complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT) and defined as the engraftment of all peripheral blood cell lines other than a platelet (PLT) count ≤2×10^10/L or dependence on PLT transfusions for more than 60 days after allo-HSCT. Several clinical risk factors have been proposed to be associated with PT after allo-HSCT. However, the underlying mechanisms remain to be elucidated. Emerging evidence from mouse studies has suggested that effective hematopoiesis depends on a particular bone marrow (BM) microenvironment in which hematopoietic stem cells reside. MSCs represent a key cellular component of the BM microenvironment, which are potential progenitors for osteoblasts, adipocytes, chondrocytes, and marrow stromal cells. The interactions of megakaryocytosis and thrombopoiesis result from the interactions between hematopoietic progenitor cells, cytokines, and marrow stromal cells derived from MSCs or MNCs directly. However, the functional role of BM MSCs in the patients with PT has never been reported. Moreover, approaches for improving the dysfunction of BM MSCs in patients with PT are lacking.

**Aims:** To evaluated the number and function of BM MSCs in patients with PT post-allo-transplant. Moreover, to investigate the approach to enhance the number and function of BM MSCs derived from patients with PT and its underlying molecular mechanisms in vitro.

**Methods:** Three cohorts were included: patients with PT (N=25), patients with good graft function (GGF, N=12), defined as persistent successful engraftment after allo-transplant, and transplant donors as normal controls (N=10). BM MSCs were cultured as previous reported. All experiments were carried out using BM MSCs derived from passages 2–4. The number and functions of BM MSCs were evaluated by fibroblasts colony-forming unit (CFU-F) assay, cell proliferation, and cell-associated-S-nitrosothiolase (SNO). Reactive oxygen species (ROS) levels were evaluated by flow cytometry. Protein expression for p-p38, p38, p-p53, p35 was measured by flow cytometry and western blots. To further investigate the potential effect for repairing the dysfunctional BM MSCs, N-Acetyl-L-cysteine (NAC) scavenger, and SB203580 (p38 inhibitor) were administrated to the BM MSCs for PT patients. After 2 days in vitro culture, the number of SAβ-positive cells was counted, the intracellular levels of ROS and p-p38 were evaluated in BM MSCs by flow cytometry.

**Results:** Human BM MSCs were demonstrated as spindle shape and typical immunophenotype of MSCs at day 21 of cultivation among subjects with PT, GGF and normal controls. Cultures from all BM samples produced confluent layers of adherent cells composed of spindled shaped cells. 2 of the 12 GGF BM and 15 of the 25 PT BM failed to produce any adherent layers within 3 weeks of culture. BM MSCs derived from PT patients expanded more slowly and appeared flattened and larger. Proliferative capacity and CFU-F counts of BM MSCs from PT patients were significantly reduced compared to those of GGF patients and normal controls. Moreover, increased levels of ROS, which was associated with increased number of SAβ-positive cells, were identified in BM MSCs from PT patients. Intracellular p-p38 level was significantly elevated in PT compared to those in GGF patients. After NAC treatment in vitro, the ROS level was increased significantly whereas the number of senescent cells, the intracellular levels of ROS and p-p38 were reduced markedly in BM MSCs from PT patients.

**Summary/Conclusions:** In summary, the current study demonstrated the number and the function of BM MSCs were abnormal in PT patients following allo-HSCT. NAC and in vitro treatment in PT patients expanded p38 MAPK pathway and reversed the senescence phenotype through down-regulation of the p38 MAPK pathway. Our results indicate that the dysfunctional BM MSCs may play an important role in the pathogenesis of PT following allo-HSCT and NAC represents a promising therapeutic approach for repairing the impaired BM MSCs in PT patients post-allo-transplant.

**P575**

**GRAFT-VERSUS HOST DISEASE (GVHD) DEVELOPMENT AFTER BONE MARROW TRANSPLANTATION IS NOT INFLUENCED BY TH9 CELLS**

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**Background:** Th9 cells are a recently defined subset of Th helper cells (Th) characterized by the massive production of IL-9. Th9 cells mediate immune responses against helminth infections, exhibit anti-tumor immunity against solid tumors and mediate allogeneic transplant tolerance but they also contribute to immunopathology in allergy and autoimmunity.

**Aims:** Currently, the role of Th9 cells for GVHD induction and the graft-versus-tumor effect is largely unknown. Therefore, we first explored, whether Th9 cells are induced during GVHD development in two different MHC-mismatched bone marrow transplantation (BMT) models and secondly analyzed, whether transplantation of in vitro-generated Th9 cells mediates GVHD.

**Methods:** We transplanted allogeneic BM and spleen cells from B6-SJL mice (CD45.1, H-2b) in B6D2F1 mice (CD45.2, H-2bd2) or in B6.Bm12 mice (CD45.2, Bm12). In the first model, we inoculated BM from cIAP—/- donor mice with H-2b naive BM and analyzed the induction of Th9 cells during GVHD development. To clarify whether in vitro-generated Th9 cells mediate GVHD, we induced Th9 cells in vitro from isolated naive CD4+ T cells with anti-CD3/CD28 coated plates by TGF-b, IL-4, anti-IFNg and recombinant TL1A and co-injected them intravenously into lethally irradiated B6 BM in irradiated recipient mice and subsequently monitored GVHD induction.

**Results:** In both MHC mismatched models used, the transplantation of allogeneic spleen cells and BM leads to GVHD characterized by a time-dependent strong increase of Th1-specific cytokines TNF-a and IFN-g in the serum of the recipient mice. Therefore, IL-9, however, was strongly elevated. When allogeneic T cells were identified in the spleen, liver and lung of GVHD-developing animals until 29 days after transplantation, while TNF-a and IFN-g producing cells were strongly increased indicating that Th9 cells are not induced

**References:**

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during GVHD. After in vitro differentiation of Th9 cells from naive T cells we obtained more than 60% of IL-9-producing cells after 5 days of culture. Th9 cells differ in their cytokine profile (IL-9+, IFN-γ-, IL-13-) from Th1 and Th2 cells. Transplantation of in vitro-generated Th9 cells together with allogeneic BM cells did not induce GVHD in the MHC-disparate recipient mice, while the transplantation of unselected T cells or in vitro-generated Th1 cells induced GVHD and resulted in death in about 60% of the animals. Although no GVHD development was detected, Th9 cells migrated into lymphoid organs and GVHD target organs such as spleen and lung. Surprisingly, when the cytokine phenotype of the transplanted Th9 cells were analyzed after ex vivo isolation from spleen and liver at different time points after transplantation, the cells lost their IL-9+ and IL-4+ after the transfer. Furthermore, to a plasticity of Th9 cells after adoptive transfer. Systemic increase of TNF-α and IFN-γ in the serum of mice receiving Th9 cells, however, was not detected.

Summary/Conclusions: Th9 cells are not induced during GVHD development and the adoptive transfer of in vitro-generated Th9 cells does not induce GVHD. However, the transplanted Th9 cells home to spleen and GVHD target organs and start to produce TNF-α and IFN-γ without strong systemic increase in these cytokines. Since TNF-α and IFN-γ are cytokines associated with an anti-tumor cytotoxicity and Th9 cells are known to eliminate solid tumors, future experiments will define whether in vitro-generated Th9 cells can be used as a cellular therapy for anti-tumor responses in BM-transplanted hosts.

Background: Co-transplantation of human mesenchymal stromal cells (hMSC) has been reported to reduce the risk of graft failure and improve hematopoietic stem cells (HSC) engraftment in xenogenic and determined allogeneic transplants. In addition, we have demonstrated that the co-infusion of MSCs with low numbers of purified HSCs significantly improve the short- and long-term hematopoietic reconstitution in an autologous HSC experimental model with splenectomy (57g). Aims: The aim of this study is to analyze the effect of MSCs on HSC engraftment in a clinically relevant model of hematopoietic gene therapy. Methods: We have studied the effect of MSCs co-infusion in a mouse model of HSC gene therapy with risk of engraftment failure in Fanconi anemia mice (Fanca−/−). Results: In these experiments, the infusion of low numbers of WT LSK cells (1,500 LSK) in Fanca−/− mice resulted in 30% graft failure, which was prevented when 6.105 Ad-MSCs were co-infused. Furthermore, when 1,500-3,000 Fanca−/− LSK cells transduced with a therapeutic lentiviral vector (PGK-FANCA-wPRE) were transplanted, the infusion of similar cell doses resulted in more than 50% of engraftment failure, which decreased to 30% only when more than 10,000 gene-corrected LSK were infused. Once again, Ad-MSCs co-infusion prevented graft failure in the infusion with the same number of gene-corrected LSK cells. Summary/Conclusions: Taken together, our results demonstrate the potential of Ad-MSCs to avoid graft failure in a clinically relevant model of hematopoietic gene therapy with risks of engraftment failure.
Although C57BL/6N (N) and C57BL/6J (J) mice are derived from the same parental C57BL/6 strain, there are key genotypic and phenotypic differences between these sub-strains. However, more than 58% of studies published involving C57BL/6 mice do not indicate the specific sub-strain employed. J mice have a five-exon deletion in the Nicotinamide nucleotide transhydrogenase (*Nnt*) gene that results in a non-functional protein. NNT is involved in the resolution of oxidative stress in the mitochondria. Hematopoietic stem cells (HSCs) can reconstitute the entire hematopoietic system after transplantation into hosts whose hematopoietic compartment has been ablated. This is clinically exploited as HSCs transplantation (HSCT) to treat hematologic diseases and represents the only curative therapy for many disorders. During HSCT, HSCs subject to oxidative stress. Short-term J-lymphoid-biased progenitors generated fewer and smaller CFU than N-HSPCs when isolated from pI:pC in HSPCs isolated from pI:pC treated J mice than N mice with the exception of increases ROS levels in HSPCs. We found about two-fold higher ROS levels in J-MPP3s and J-MPP4s. It is known that pI:pC treatment elevates ROS post-transplant.

**Aims:** As elevated oxidative stress compromises hematopoietic stem and progenitor cell (HSPC) function, here we thoroughly interrogated the frequency and function of HSPCs in J and N bone marrow (BM).

**Methods:** N and J peripheral blood (PB) and BM (n=9) was interrogated by flow cytometry for the absolute frequencies of all major hematopoietic lineages and HSPC compartments, respectively. 5000 J or N CD45.2 HSPCs (Lin-Sca-1+c-Kit+ cells) were transplanted along with 5000 competitor CD45.1 HSPCs into lethally irradiated mice to test for competitive in vivo hematopoietic repopulating activity and ROS levels post-transplant. Lineage potential and reconstituting activity of multi-potent progenitors (MPP2: Lin-Sca1+ c-Kit+Flt3-CD48+CD150+, MPP3: Lin-Sca1+ c-Kit+Flt3-CD48+CD150-), MPP4: Lin-Sca1+ c-Kit+Flt3+CD48+CD150- was also tested by transplanting 2000 MPPs from J or N mice into sub-lethally irradiated mice and examining the PB of recipients every 3-4 days for 34 days post-transplant. Sensitivity of HSPCs to oxidative stress was tested by examining ROS levels and the in vivo colony forming unit (CFU) potential of HSPCs isolated from N and J mice treated with pI:pC.

**Results:** The frequency of the major PB lineages and bone marrow HSPC compartments was identical in J and N mice. However, J-HSPCs displayed compromised short-term (4-12 weeks post-transplant) hematopoietic repopulating activity relative to N-HSPCs that was driven by a delay in lymphoid reconstitution. No differences were found in donor contribution to bone marrow HSPC compartments at 20 weeks post-transplant. However, donor-derived MPPs and CLPs displayed a two-fold increase in ROS levels in recipients of J-HSPCs versus N-HSPCs at 20 weeks post-transplant. MPPs are responsible for repopulation of the hematopoietic system during this early window post-transplant. Different MPP subpopulations can be defined (MPP2, MPP3 and MPP4) according to their self-renewal potential and specific lineage potential. MPP3s and MPP4s are the first MPP subpopulations to reconstitute the lymphoid lineage after transplant. J-MPP3s and J-MPP4s displayed less in vivo repopulating activity than N-MPP3s and N-MPP4s. It is known that pI:pC treatment increases ROS levels in HSPCs. We found about two-fold higher ROS levels in HSPCs isolated from pI:pC treated J mice than N mice with the exception of the myeloid progenitor compartments (CMP, GMP and MEO). J-HSPCs also generated fewer and smaller CFU than N-HSPCs when isolated from pI:pC treated mice. These data indicate that J-HSPCs cannot resolve oxidative stress as efficiently as N-HSPCs, which may be due to lower self-renewal potential after exposure to oxidative stress. Short-term J-lymphoid-biased progenitors (e.g. MPPs and CLPs) were especially sensitive to increasing ROS, which very likely drives the short-term loss of in vivo repopulating activity.

**Summary/Conclusions:** Based on these data, we hypothesize that loss of the *Nnt* gene in C57Bl/6J mice sensitizes HSPCs to oxidative stress, which compromises their short-term in vivo hematopoietic repopulating activity.

**Thrombosis disorders**

**P759**

**GWAS RESULTS IN RED BLOOD CELL PHENOTYPES AND THEIR RELATIONSHIP WITH THROMBOSIS**


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**Background:** Venous thromboembolism (VTE) is a complex and multifactorial disease with a estimated heritability of 60%. Intermediate phenotypes of VTE have been used to identify genetic risk factors. We previously reported a genetic correlation of 5 erythrocyte phenotypes with VTE.

**Aims:** To identify single nucleotide polymorphisms (SNPs) influencing the phenotypic variance of erythrocyte parameters, especially those related to VTE, in Spanish families from the Genetic Analysis of Idiopathic Thrombophilia (GAIT2) Project.

**Methods:** Genome-wide association analyses (GWAS) with ~10M SNPs were performed for eighteen erythrocyte phenotypes in 935 subjects belonging to 35 extended families with thrombosis of GAIT2. The erythrocyte phenotypes evaluated included: Hemoglobin (Hb), red blood cell count (RBC), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), reticulocyte (RET), low fluorescence reticulocyte (LFR), middle fluorescence reticulocyte (MFR), high fluorescence reticulocyte (HFR), reticulocyte fluorescence index (RFI), haptoglobin (HP), serum iron (Fe), total iron binding capacity (TIBC), saturation index (SI), serum ferritin (FT) and serum transferrin receptor (TFR).

**Results:** We identified 12 SNPs showing association with the 5 erythrocyte phenotypes previously related to VTE (Table 1). Interestingly, the rs56306145 that showed association with TFR is an intronic variant located in the gene tissue factor pathway inhibitor 2 (TFPI2), which encodes a protein that inhibits a variety of serine proteases of blood coagulation, such as activated factor VII (FVIIa/TF), FXa, plasmin and plasma kallikrein. These data reinforce our previous report of genetic correlation of TFR with VTE. The most significant SNP-associations were reported.

**Table 1. Top SNP-associations with erythrocyte phenotypes related to VTE from GWAS in GAIT2.**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>SNP</th>
<th>Chr</th>
<th>Type</th>
<th>Closest gene</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>rs449</td>
<td>11</td>
<td>Intron</td>
<td>ANO5</td>
<td>1.0E-06</td>
</tr>
<tr>
<td>RDW</td>
<td>rs961805</td>
<td>20</td>
<td>Intronic</td>
<td>TFPI2</td>
<td>2.0E-12</td>
</tr>
<tr>
<td>MCH</td>
<td>rs977270</td>
<td>3</td>
<td>Intron</td>
<td>MAF</td>
<td>6.4E-08</td>
</tr>
<tr>
<td>SAT</td>
<td>rs600155</td>
<td>17</td>
<td>Intronic</td>
<td>TFPI2</td>
<td>8.7E-07</td>
</tr>
<tr>
<td>TFR</td>
<td>rs519564</td>
<td>2</td>
<td>Intron</td>
<td>TFPI2</td>
<td>3.1E-08</td>
</tr>
</tbody>
</table>

**Reference:**


This work was supported by RIC RD12/00420032, FIS PI12/00612 and FIS PI15/0269 grants.
Background: ET and PV are characterized by a high incidence of arterial and venous thrombosis. Platelet (PLT) count is not an independent risk factor for thrombosis in these conditions. However, no information is available on patient PLT qualitative properties, i.e., the PLT thrombus formation capacity in a dynamic condition.

Aims: We wanted to evaluate, in a group of ET and PV patients, the PLT thrombus formation capacity by an ex-vo-vivo dynamic model of PLT adhesion under flow conditions, and to establish the influence of JAK2-V617F/Calreticulin (CalR)/MPL mutations, hematological parameters, and ongoing therapies.

Methods: One hundred-thirty patients, i.e., 78 ET (32 M/46 F; median age=61 years, range 28-86) and 52 PV (26 M/26 F; median age=65 years, range 38-78) were enrolled after informed consent. For the adhesion assay, peripheral venous whole blood was drawn in sodium citrate, recalculated in the presence of heparin, and perfused over a collagen-coated surface for 4 min. at a shear rate of 1,000 s⁻¹. PLTs were then stained with an anti-CD62P (P-selectin)-FITC antibody to evaluate PLT activation, and annexinV-AlexaFluor647 to detect pro-coagulant phosphatidylserine expression. After staining, phase contrast and fluorescence images of adherent PLTs were taken in random fields using an EVOS® microscope. Results are expressed as the means(SEM) of the % of area covered by all PLTs (% coverage), or as the % of adherent PLTs positive for pro-coagulant phosphatidylserine expression. Main hematological parameters, therapies, and mutational status were recorded.

Results: PLT adhesion was significantly (p<0.01) greater in either ET (45.3±1.7%) and PV patients (48.9±1.6%) compared to healthy controls (37.5±2.7%), while no difference was found between ET and PV patients. The analysis of the mutational status shows that ET PLT adhesion was highest in JAK2-V617F mutation carriers (n=41; coverage: 47.7±2.4%, p<0.001 vs controls), followed by CalR-positive patients (n=21; coverage: 45.5±3.2%, p<0.05 vs controls, p=n.s. vs JAK2-V617F), while, PLT adhesion of MPL-positive (n=3; coverage: 32.1±1.0%) or triple negative (n=13; coverage: 42.6±2.5%) ET patients was not statistically different from controls. In PV, no statistically significant difference was observed between subjects with >50% versus those with <50% JAK2-V617F allele burden. According to treatment, we observed that ET patients treated with the combination of aspirin+hydroxyurea presented the lowest PLT adhesion, while in PV no significant difference was observed between different antiplatelet or phosphatidylserine stay-inhibitors. Main hematological parameters, therapies, and mutational status were recorded.

Summary/Conclusions: ET and PV/platelets show an increased PLT thrombus formation potential, particularly in patients carrying the JAK2-V617F mutation. On the basis of these results, it is worth to include a dynamic PLT adhesion assay in risk prediction models to evaluate the predictive value of thrombotic events in ET and PV patients. [Project funded by “AIRC-IG2013” grant Nr. 14005 of the “Italian Association for Cancer Research” (A.I.R.C.)].

P761
DOAC ASSOCIATED MAJOR GASTROINTESTINAL BLEEDING: REAL LIFE EXPERIENCE FROM A UNIVERSITY TEACHING HOSPITAL, UK
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Background: The risk of major GI bleeding in our cohort of over 2500 patients over 3 years was noted to be significantly lower than trial data. Since this is a retrospective review from patient hospital database there is a risk of reporting bias and under-reporting of bleeding events. A prospective phase IV study to identify bleeding risk in patients on DOAC is required. Majority of patients with major GI bleeding had other risk factors such as concurrent use of anti-platelets, peptic ulcer disease, alcohol abuse, oesophageal varices, diverticular disease, and bowel malignancy which would increase their bleeding risk on any anticoagulation. Further sub group analysis of this cohort and efforts to improve reporting of anticoagulation associated bleeding is underway.

Method: One hundred-thirty patients, i.e., 78 ET (32 M/46 F; median age=61 years, range 28-86) and 52 PV (26 M/26 F; median age=65 years, range 38-78) of a major GI bleeding equating to annual risk of major GI bleeding of 0.23% per patient/year. This is a much lower incidence than reported data from clinical trials in patients on DOAC. Patients who suffered from a major GI bleeding did so on average 143 days (range 8-576) after starting the DOAC. Of all patients with major GI bleeding, 14 were taking apixaban (0.8% of all pt on apixaban), 3 (0.4% of all pt on rivaroxaban) rivaroxaban and 1 (0.3% of all pt on dabigatran) on dabigatran. The numbers were too small to identify any statistical difference between the different DOAC drugs.

Summary/Conclusions: The risk of major GI bleeding in our cohort of over 2500 patients over 3 years was noted to be significantly lower than trial data. Since this is a retrospective review from patient hospital database there is a risk of reporting bias and under-reporting of bleeding events. A prospective phase IV study to identify bleeding risk in patients on DOAC is required. Majority of patients with major GI bleeding had other risk factors such as concurrent use of anti-platelets, peptic ulcer disease, alcohol abuse, oesophageal varices, diverticular disease, and bowel malignancy which would increase their bleeding risk on any anticoagulation. Further sub group analysis of this cohort and efforts to improve reporting of anticoagulation associated bleeding is underway.

P762
Abstract withdrawn.

P763
INCIDENCE OF VENOUS THROMBOEMBOLISM IN PATIENTS UNDERGOING LOWER LIMB SURGICAL REvascularization: IS THROMBOPROPHYLAXIS WARRANTED?
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1Staffordshire Thrombosis & Anticoagulation Centre, 2Haematology, Royal Stoke University Hospital, Stoke on Trent, United Kingdom

Background: The incidence of postoperative deep vein thrombosis (DVT) or consequential pulmonary embolism (PE) in patients undergoing lower extremity surgical revascularization procedures is not well studied. The need for routine anticoagulation for DVT/PE prophylaxis after the lower limb surgical revascularization remains controversial.

Aims: The purpose of this study is to retrospectively evaluate the incidence of postoperative DVT/PE in patients undergoing lower limb surgical revascularization.

Methods: Charts for patients undergoing lower limb surgical revascularization, from 01/01/2010 to 12/31/2015, were evaluated for DVT/PE. DVT/PE within three months of the revascularization was considered to be a postoperative DVT/PE. Patients undergoing multiple procedures were counted as different cases if they were on different days. Multiple procedures on a patient on the same day were considered a single case. Patients with hypercoagulable states or previous history of DVT were excluded. Descriptive statistics and t-test was used to analyze incidence of DVT/PE and assess the importance of postoperative thromboprophylaxis.

Table 1.

<table>
<thead>
<tr>
<th>Procedure performed</th>
<th>DVT/PE within three months from surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Balloon angioplasty</td>
<td>15</td>
</tr>
<tr>
<td>Bypass aorta-femoral</td>
<td>18</td>
</tr>
<tr>
<td>Bypass femoral-peroneal</td>
<td>3</td>
</tr>
<tr>
<td>Bypass femoral-femoral</td>
<td>12</td>
</tr>
<tr>
<td>Bypass femoral-polpitle</td>
<td>5</td>
</tr>
<tr>
<td>Bypass femoral-tibial</td>
<td>6</td>
</tr>
<tr>
<td>Lower limb embolectomy</td>
<td>3</td>
</tr>
<tr>
<td>Femoral artery exploration</td>
<td>6</td>
</tr>
<tr>
<td>Thrombectomy</td>
<td>39</td>
</tr>
<tr>
<td>Total</td>
<td>354</td>
</tr>
</tbody>
</table>

Results: Between 1/1/2010 to 12/31/2015, 360 patients were found to have undergone lower extremity surgical revascularization. Study population included 200 males and 160 females. Mean patient age was 69.54 years. One patient had a previous history of DVT and was excluded. Overall, of the 359 patients, five (1.4%) were recognized to have a new DVT/PE within 3 months of the surgery. One patient developed DVT in the contralateral limb, and one developed it in the arm. Patients were recognized to have a new DVT/PE, on an average, at 7.6 days after the surgery. A one sided t-test demonstrated that the average
postoperative day for recognition of DVT/PE was significant greater than 3.5 (7.6 vs 3.5, 5.1value=2.17, p=0.048). Patients developing DVT/PE did not differ by obesity or age when compared with non-DVT/PE population.

Summary/Conclusions: There have been only a few studies to assess the incidence of DVT/PE in patients undergoing lower limb surgical revascularization. In our study population, 1.4% of patients had evidence of DVT/PE. This constitutes a low risk of venous thromboembolism. The 2012 American College of Chest Physicians (ACCP) guidelines for prevention of venous thromboembolism in nonorthopedic surgical patients (Chest 2012; 141(2)Suppl:e227s-e277s), recommends the use of pneumatic compression devices (PCDs), over no prophylaxis, to prevent DVT/PE in low risk patients. Since, patients with lower limb surgeries are not a good candidate for PCDs, pharmacological thromboprophylaxis with low dose heparin may be warranted. Given that bleeding is a potential complication in these patients, it might be prudent to start thromboprophylaxis 3.5-4 days after the surgery. Further studies are needed to assess the bleeding risks of postoperative thromboprophylaxis after surgical revascularization procedures.

Table 1. Values, heritabilities, household effect and significant covariates effects.

<table>
<thead>
<tr>
<th>TRAIT</th>
<th>Value</th>
<th>b²</th>
<th>p value (b)</th>
<th>c²</th>
<th>Covariable</th>
</tr>
</thead>
<tbody>
<tr>
<td>B12 (mmol/L)</td>
<td>4.41±0.71 (4.74±5.58)</td>
<td>0.47</td>
<td>2.95 x 10⁻²</td>
<td>0.11</td>
<td>Age, contracep, smoking</td>
</tr>
<tr>
<td>SF (mmol/L)</td>
<td>2.14±0.7 (0.2-1.31)</td>
<td>0.27</td>
<td>2.3 x 10⁻³</td>
<td>0.07</td>
<td>Sex, contracep, smoking</td>
</tr>
<tr>
<td>RCF (mmol/L)</td>
<td>1241±84.1 (957-3556)</td>
<td>0.42</td>
<td>1.85 x 10⁻³</td>
<td>0.06</td>
<td>Age, sex, smoking</td>
</tr>
<tr>
<td>Hcy (mmol/L)</td>
<td>10.6±5.5 (2.7-9.7)</td>
<td>0.36</td>
<td>3.61 x 10⁻¹</td>
<td>0.41</td>
<td>Age, sex, smoking</td>
</tr>
</tbody>
</table>

Values expressed as Mean±standard deviation, in brackets maximum and minimum values. B12: serum vitamin B12; SF: Serum folate; RCF: Red cell folate; HCY: Homocysteine.

Table 2. Suggestive signals detected by GWAS.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Chromosome</th>
<th>Gene and SNP</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>B12</td>
<td>10</td>
<td>VIF2 rs812546</td>
<td>1.74 x 10⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MTHFR rs1801133</td>
<td>1.4 x 10⁻³</td>
</tr>
<tr>
<td>SF</td>
<td>12</td>
<td>MTHFR rs1801133</td>
<td>1.34 x 10⁻⁴</td>
</tr>
<tr>
<td>RCF</td>
<td>9</td>
<td>MTHFR rs1801133</td>
<td>1.59 x 10⁻⁴</td>
</tr>
<tr>
<td>HCY</td>
<td>9</td>
<td>MTHFR rs1801133</td>
<td>2.4 x 10⁻⁶</td>
</tr>
</tbody>
</table>

Summary/Conclusions: In the GAIT2 study, genetic and environmental factors were related to B12, SF, RCF and HCY. Moreover, a relationship was observed between B12 and VTE. In the GWAS analysis some signals were previously reported (FUT2 and B12 or MTHFR with SF and HCY). New signals were found that need to be clarified, especially their possible relationship with susceptibility to thrombosis.

This work was supported by RIC RD12/0042/0032, FIS PI12/00612 and FIS PI 15/0269 grants.

P766

CELLULAR ORIGIN OF CIRCULATING MICROPARTICLES (MP) ACCORDING TO SOMATIC MUTATIONS IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS (MPN)

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Background: Essential thrombocythemia (ET) and polycythemia vera (PV) are MPN characterized by a high rate of thrombotic complications. We previously demonstrated increased plasma levels of procoagulant MP in ET (Marchetti et al. A.J.H. 2013).

Aims: Aim of this study was to extend the analysis of MP to PV patients and to characterize the cellular origin of plasma MP in both ET and PV patients. The influence of somatic mutations [i.e. JAK2V617F, calreticulin (CaIR), thrombopoietin receptor (MPL)] and concomitant cytoreductive or antiplatelet therapies was also evaluated.

Methods: Thirty-seven ET (19 JAK2V617F, 9 CaIR and 2 MPL mutation carriers), 35 PV patients (all JAK2V617F carriers) and 36 healthy control subjects were included into the study. Flow cytometry was performed to characterize MP phenotype in platelet free plasma samples. To define MP cellular origin, anti-CD31 (endothelial cell marker), anti-CD41 (platelet marker), anti-CD11b (leucocyte marker), and anti-CD235 (erythrocyte marker) monoclonal antibodies were used. Annexin V (AnnV) staining was used to evaluate the expression of procoagulant phosphatidylserine on MP.

Results: ET and PV patients displayed significantly higher MP levels compared to controls (p<0.05). The majority of circulating MP (90%) were AnnV positive, indicating the expression of phosphatidylserine on their surface. In healthy con-
trols, 71% of MP was positive for platelet (P-MP), 24% for erythrocyte (E-MP), 4% for endothelial cell (EC-MP) and 1% for leukocyte (L-MP) specific markers. In ET and PV patients, the percentage of P-MP was significantly higher (80%; p<0.05), while E-MP level was significantly lower (15%; p<0.05) than controls. L-MP and EC-MP values were comparable between patients and controls. The absolute counts of P-MP and L-MP were higher in both ET and PV versus controls. Overall, no significant correlations were found between the levels of MP derived from platelet, leukocytes or erythrocytes and the corresponding cell counts. The analysis according to patient mutations, revealed significantly higher levels (p<0.05) of both P-MP and E-MP concentration in patients carrying JAK2V617F mutation as compared to JAK2V617F negative patients. In addition, ET patients positive for CaR mutation displayed lower levels (p<0.05) of P-MP compared to JAK2V617F carriers. No influence of concomitant therapies on MP levels or composition was observed.

Summary/Conclusions: Our data confirm the presence of high levels of circulating MP in MPN, which support the role in the known hypercoagulable state of these patients. The MP cellular origin has a different distribution profile according to the presence of different mutations. Importantly, the lack of correlation found between the total and subtype-specific MP counts with the corresponding cell of origin suggests an active stimulation of MP formation.

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P767
ARE WE TESTING APPROPRIATELY FOR THE LUPUS ANTICOAGU-LANT?
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Background: The diagnosis of antiphospholipid syndrome (APS) requires the presence of thrombosis or defined pregnancy morbidity in addition to the presence of antiphospholipid antibodies on at least 2 occasions. Patients should be tested for antiphospholipid antibodies if they fulfil the required clinical criteria. Lupus anticoagulant may also be tested for when investigating a prolonged activated partial thromboplastin time which does not correct on mixing studies. Aims: The aim of our study was to examine retrospectively the frequency of lupus anticoagulant (LA) testing in our institution, which we suspected to be high, and the incidence of positive results leading to a diagnosis of APS.

Methods: A total of 914 requests for LA were received over a 5 month period between 1st of May and 30th September 2014. We examined which departments were requesting the tests and the clinical indications for testing.

Results: Over 90% (829) of LA tests were negative. Nine percent (85) of tests demonstrated a positive LA. 33 patients had experienced arterial (11) or venous (22) thrombosis. There were 3 patients who fulfilled the clinical criteria for pregnancy morbidity in APS. A total of 6 patients experienced miscarriage before 10 weeks gestation; however none of these patients had the defined 3 miscarriages. There was one preterm delivery at 25 weeks due to pre-eclampsia. A further 3 patients had a still birth, one of which had an identifiable cause. In total, of the 85 positive results, 12 patients had a confirmed diagnosis of APS; a further 25 patients had the clinical manifestations fitting the clinical criteria for APS. Forty eight patients had a positive LA but did not fit the clinical criteria for a diagnosis of APS. The clinical specialties requesting the majority of tests were obstetrics and gynaecology (231), rheumatology (179) and clinical haematology (110). Of these, clinical haematology had the highest yield of positive results (16%) compared to 3% in obstetrics and gynaecology.

Summary/Conclusions: Our results highlight a high frequency of LA testing in our institution with a low yield of positive results (9%), resulting in a total of 1% of patients being diagnosed with APS. Our results demonstrate that the majority of tests for LA are not of clinical significance and often requested in patients not fitting the clinical criteria for APS. Further education for all practitioners would help to ensure only appropriate patients are tested. Indeed if a patient fits the clinical criteria for APS they should be tested for all antiphospholipid antibodies namely anti-cardiolipin and anti-β2-glycoprotein I as well as the lupus anticoagulant.

P768
RESULTS OF USING BRIDGING THERAPY WITH SODIUM BEMIPARIN AT THERAPEUTIC-DOSE
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1Servicio de Hematologia y Hemoterapia, Complejo Hospitalario Universitario de Granada, Granada, 2Unidad de Cuidados Intensivos, Complejo Asistencial de Soria, Soria, Spain

Background: Bridging therapy consists of the administration of a fast-acting anticoagulant such as the low-molecular-weight heparin (LMWH) during the period of cessation of oral anticoagulant therapy. The decision to continue with anticoagulant therapy or to discontinue the treatment with the establishment of the Bridging therapy have been carried out carefully and on an individual basis.

While taking this decision, we have taken into account three factors: the urgency of surgery or invasive process, the risk of bleeding and thrombotic risk for the patient. In recent decades, there have been multiple studies supporting the LMWH treatment, at least as safe and effective and more cost-effective than unfractionated heparin (UHF) in the 6-8 days of venous thromboembolism (VTE), including deep vein thrombosis (DVT) and pulmonary embolism (PE). Therefore, the LMWH is considered as the drugs of choice in the prevention of venous thromboembolism.

There are several types of commercialized LMWH, with different pharmacological properties, such as molecular weight, anti-Xa/IIa ratio and average life. The sodium bemiparin is the LMWH with greater anti-Xa/IIa ratio, which implies a lower risk of bleeding. In addition, it has shown a low incidence of VTE and bleeding in actual clinical practice.

Aims: There are few published data from bridging therapy at therapeutic doses in patients treated with oral anticoagulants (AVK) and perioperative management. It is intended to assess the efficacy (recurrence of thrombosis) and safe use of sodium bemiparin at anticoagulant doses on the bridging therapy and possible thrombotic and/or hemorrhagic complications (major and minor bleeding) resulting from this use.

Methods: We have analyzed 975 bridging therapies at full dose in our clinic in the last year. They were made to a total of 650 patients (315 men and 335 women) with CHADS2/VASC >2, aged between 15 and 92, with an average age of 69 years old. The reasons of anticoagulation in our patients were atrial fibillation, mechanical prostheses, DVT, pulmonary embolism and recurrent thrombosis in patients with thrombophilia. In 70% of the cases, there were comorbidities, such as heart failure, chronic obstructive pulmonary disease, anemia, kidney failure, liver disease and long-term aftereffects of stroke. The bridging therapy has consisted on suspending the AVK (115 IU/kg/24h) to 6 days (warfarin) before the procedure, and replacing it by sodium bemiparin at full doses <50 kg: 5.000 IU/24h, 50 to 70 kg: 7.500 IU/24 h, 70-100 kg: 10.000 IU/24 h and >100 kg: 12.500 IU/24 h, and administration of a prophylactic dose of 3.500 IU, 12 hours before the procedure, and another dose 6-12 hours after the procedure, depending on the risk of bleeding of the intervention and the thrombotic risk of the patient’s disease. The bridging therapy has been performed in 225 cases of major surgery (orthopedic surgery, ophthalmological procedures, valvar replacements etc.), 340 cases of minor surgery (removal of nevus, complex dental extractions, dental implants), 295 cases of invasive procedures (colonoscopies, endoscopies...), 50 cases of bleeding caused by AVK (epistaxis, petechiae and bruises, hemoptysis, menorrhagia and gastrointestinal bleeding), 30 cases of hospitalization with INR decompensation with various causes (infectious endocarditis, pneumonia, uncompensated heart failure...) and 35 cases for thrombophilia study.

Results: As complications of using bemiparin sodium, there have been: 40 cases of hematomas at the needle puncture sites. There was neither cases of major bleeding nor cases of thrombosis.

Table 1.

Summary/Conclusions: Sodium bemiparin administered at therapeutic doses (115 IU/kg/24h) in the perioperative period, according to the scheme described above, is associated with a low incidence of recurrence of VTE and bleeding. The complications presented in our sample have been very few, in patients with associated co-morbidities. In our study, sodium bemiparin has shown to be safe and effective with minimal bleeding complications. Treatment should be administered on an individual basis according to each patient and factors related to surgery. Further studies will confirm our results.
Targeted therapies in relapsed in chronic lymphocytic leukaemia

**S769**  
**IBRUTINIB IN PREVIOUSLY TREATED CHRONIC LYMPHOCYTIC LEUKAEMIA: UPDATED EFFICACY AND SAFETY OF THE RESONATE STUDY WITH UP TO FOUR YEARS OF FOLLOW-UP**


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**Background:** Ibrutinib is a first-in-class, once-daily oral inhibitor of Bruton’s tyrosine kinase. Ibrutinib as a single agent is indicated by the EMEA and US FDA for the treatment of adult patients with CLL and allows for treatment without chemotherapy. The phase 3 RESONATE study in patients with relapsed CLL showed superior efficacy of ibrutinib compared with ofatumumab (Byrd NEJM 2014).

**Aims:** We report updated safety and efficacy results of the RESONATE trial with up to 4 years of follow-up.

**Methods:** Eligibility criteria included ≥1 prior therapy, ineligibility for treatment with a purine analog, and ECOG performance status 0-1. Informed consent was obtained from all patients prior to study initiation. Patients received oral ibrutinib (420 mg once daily) until disease progression or unacceptable toxicity or intravenous ofatumumab (300 mg week 1; 2000 mg weekly for 7 weeks and then every 4 weeks for 16 weeks) for up to 24 weeks. At the interim analysis (median follow-up of 9 months), the data monitoring committee declared superiority of ibrutinib vs ofatumumab for progression-free survival (PFS) and overall survival (OS), and access to ibrutinib was recommended for all patients in ofatumumab arm who had disease progression. Long-term follow-up of efficacy endpoints are ongoing. Patients randomized to ofatumumab were censored at crossover for OS.

**Results:** Patients were randomized to receive ibrutinib (n=195) or ofatumumab (n=196). The median age was 67 years, with 40% age ≥70 years, and Rai stage III/IV in 57% of patients. At a median follow-up of 44 months (maximum 53 months) for the ibrutinib arm, PFS was significantly longer for ibrutinib vs ofatumumab (median NR vs 8 months, [HR 0.133; P<0.0001]). The 3-year PFS was 59% for ibrutinib vs 3% for ofatumumab. A significant PFS benefit was observed across baseline subgroups. In the ibrutinib arm, PFS for the del11q subgroup trended to have the most favorable outcome; however, PFS outcomes were not statistically different for patients with del17p or del11q or patients without these FISH abnormalities. At time of analysis, with the majority of patients randomized to ofatumumab (68%) crossing over to receive ibrutinib therapy, OS was longer for ibrutinib vs ofatumumab (median OS NR for either arm). The 3-year OS rate for ibrutinib was 74%. The ORR for ibrutinib was 91% with a CR/CRi rate that increased over time (currently 9%). Baseline cytopenias improved with extended ibrutinib therapy for hemoglobin (85% of patients), platelet (95% of patients), and absolute neutrophil counts (95% of patients). The adverse event (AE) profile of ibrutinib was consistent with previous reports. During a follow-up of up to 4 years, major hemorrhage occurred in 6%, grade 3 atri atrial fibrillation occurred in 6%, and grade 3 hypertension occurred in 8% of patients. The incidence of most grade ≥3 AEs decreased from year 1 vs year 2-3: neutropenia: 18% vs 6%; pneumonia: 11% vs 4%; atrial fibrillation or AV block respectively. The most frequent reasons for treatment discontinuation were progressive disease (27%) and AEs (12%). At analysis, 90 patients randomized to ibrutinib (46%) continue to receive ibrutinib.

**Summary/Conclusions:** In this international phase 3 RESONATE study with median follow-up of up to 4 years, long-term treatment with ibrutinib showed a favorable tolerability profile with sustained PFS and OS benefit regardless of high-risk cytogenetics. The results in relapsed del17p and del11q patients compared favorably to those previously reported in phase 2 studies.

**S770**  
**THE INITIAL REPORT OF THE BLOODWISE TAP CLARITY STUDY COMBINING IBRUTINIB AND VENETOCLAX IN RELAPSED, REFRACTORY CLL: SWISS ACCESSible SAFETY AND PROMISING EARLY INDICATIONS OF EFFICACY**

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**Background:** Ibrutinib (IBR) is an oral BTK inhibitor with high response rates in CLL. Venetoclax (VENOCLAX) is a potent, highly selective, orally bioavailable small-molecule BCL2 inhibitor. Both IBR and VEN are approved by the FDA and EMA as single agents for chronic lymphocytic leukaemia (CLL). IBR leads to a rapid nodal response with re-distribution of CLL into the peripheral blood whereas VEN leads to depletion of CLL cells to levels in some patients where they cannot be detected. Two of the key cellular processes that are abnormal in CLL are proliferation and apoptosis. The combination of IBR with VEN is therefore logical as biological the two drugs would be expected to be synergistic. The eradication of minimal residual disease (MRD) from blood and bone marrow is associated with improved outcome in any treatment of CLL where it has been reported.

**Aims:** The CLARITY trial (ISCRTNT: 13751882) is a feasibility study to investigate the safety and efficacy of IBR combined with VEN in patients with relapsed/refractory CLL. Here we report for the first time the safety of the combination as well as early signs of potential synergy.

**Methods:** After 8 weeks of IBR monotherapy (420mg/day), VEN was added at a dose of 10mg/day with weekly escalations to 20mg, 50mg, 100mg, 200mg to a final dose of 400mg/day. After the initial 3 patients when there was no sign of tumour lysis syndrome (TLS) the starting dose of VEN was amended to 20mg/day. The primary end-point of the trial is MRD eradication (defined as less than 1 CLL cell in 10,000) in the bone marrow after 12 months of IBR+VEN. Key secondary end-points are MRD eradication from the bone marrow after 6 and 24 months of combined IBR and VEN as well as the safety of the combination. Important safety events that were considered critical were the incidence of laboratory and clinical TLS. All patients were given prophylactic treatment with uric acid reducing agents beginning at least 72 hours prior to their initial dose of VEN. Over the first three months of combined therapy the level of CLL in the peripheral blood was monitored weekly during VEN escalation and then monthly thereafter. 50 participants will be treated in total.

**Results:** A total of 35 patients have been recruited between May 2016 and January 2017. To date 21 patients have completed the dose escalation period of IBR+VEN in combination with IBR. To date there has been only a single case of laboratory TLS in a patient whose phosphate (1.21 to 1.48 mmol/l) and creatinine (75 to 146 umol/l) both increased when VEN was increased from 100mg to 200mg. Dosing of VEN was interrupted for 7 days (due to the logistics of clinic closure periods over the Christmas break) and IBR for 24 hours. The biochemical changes were resolved with dose reduction and the patient was continued on 400mg/day of VEN with no further TLS. As yet there have been a total of 5 SAES and 22 AE’s of special interest with notably lung infection (n=3) and neutropenia (n=11) occurring on more than one occasion. All SAES’s resolved with appropriate management and all patients remained on therapy. No SUSAR’s have been reported and no AE’s have been fatal. The level of CLL in the peripheral blood increased during the 8 weeks of IBR monotherapy at 420mg/day from a median of 50 x 10^9/l (range: 0 to 330) to 55 x 10^9/l (range: 0 to 237) and then fell during the first 8 weeks of combined IBR with VEN (4 weeks dose escalation followed by 4 weeks at 400mg/day) from a median of 55 x 10^9/l to a median of 0.017 x 10^9/l (range: 0 to 3.1). The rate of fall is rapid in all patients with a median of 3 log reduction in CLL level after 8 weeks of combined therapy.

**Summary/Conclusions:** The combination of IBR with VEN is well tolerated in relapsed, refractory CLL with to date only a single case of laboratory TLS. The dose escalation period in the combination of IBR and VEN in the first phase of VEN with IBR is promising and suggests a potent synergy between the drugs. The initial bone marrow responses are expected after 6 months of combination therapy.
Background: Venetoclax monotherapy in patients (pts) with relapsed/refractory CLL harboring deletion 17p (del17p) resulted in an ORR of 79% with a CR rate of 11%, with 12-month PFS and OS estimates of 50% and 72%, respectively. The primary aim of the study was to understand the safety and activity of a novel anti-CD20 mAb+PI3Kδ+BTK inhibitor (ibrutinib) in pts with B-cell malignancies.

Methods: Eligible pts had CLL or rel/ref NHL w/o limit to prior therapies, including those ref to prior PI3Kδ or BTK inhibitors. UTX dosed on D 1, 8, 15 of C 1, D 1 of C 2-6, and C 9 & 12. TGR-1202 dose escalated (400/600/800mg QD), ibrutinib dosed at 420mg (CLL) or 560mg (NHL), both on C1D1.

Table 1.

<table>
<thead>
<tr>
<th>Subtype</th>
<th>CR</th>
<th>PR</th>
<th>ORR</th>
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<tbody>
<tr>
<td>CLL/SLL</td>
<td>19</td>
<td>3</td>
<td>100%</td>
</tr>
<tr>
<td>FL/MZL</td>
<td>6</td>
<td>2</td>
<td>86%</td>
</tr>
<tr>
<td>DLBCL</td>
<td>0</td>
<td>4</td>
<td>100%</td>
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<tr>
<td>MCL</td>
<td>4</td>
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53% of evaluable CLL pts had high-risk cytogenetics and 4/6 DLBCL pts were non-GCB. One CLL pt (17p/11q del) ref to PI3Kδ and ibrutinib achieved a CR.

Summary/Conclusions: This is the first known triplet combination of an anti-CD20 mAb+PI3Kδ+BTK inhibitor. The combination of UTX, TGR-1202, and ibrutinib has been well tolerated with activity observed across heavily pre-treated advanced B-cell malignancies. Expansion cohorts at the highest dose (800mg TGR-1202+full dose ibrutinib) are underway. Future trials for the triplet are warranted.

S772

CHEMO-FREE TRIPLET COMBINATION OF TGR-1202, UBLITUXIMAB, AND IBRUTINIB IS WELL TOLERATED AND HIGHLY ACTIVE IN PATIENTS WITH ADVANCED CLL AND NHL


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Background: Subsets of B cell malignancies are addicted to B cell antigen receptor (BCR) signaling for survival. Co-stimulation of the BCR with IL-2 or IL-4 in normal B cells significantly enhances cellular activation relative to BCR or cytokine stimulation alone, and combining SYK selective and JAK selective inhibitors synergize to suppress this response (Coffey et al., 2013). Hence, BCR/SYK and cytokine JAK/STAT signals cooperate to control B cell activation. This cooperation appears to be relevant to B cell malignancies as well. IL-4 promotes the survival of CLL cells in culture via up-regulation of MCL1 and BCL(XL), protecting the tumor from death induced by fludarabine and chlorambucil (Steele et al., 2010) and by ibrutinib and ibritinib (Aguilar-Hernandez et al., 2016). Also, unlike ibrutinib, combined SYK and JAK inhibition by cerdulatinib induces apoptosis in primary CLL cells and leads to down-regulation of MCL1 and BCL(XL) (Blunt et al., 2015) and induces apoptosis in cells from ibrutinib-resistant CLL patients (Guo et al., 2017). It also induces apoptosis in primary DLBCL and DLBCL cell lines that carry BCR pathway mutations resistant to ibrutinib (Ma et al., 2015) and combined SYK/JAK inhibition may therefore represent a powerful strategy to control B cell malignancies. A phase I dose escalation study of cerdulatinib in 43 patients with relapsed/refractory CLL and NHL was recently completed (Hamlin et al., EHA Congress 2016). Inhibition of both BCR/SYK and JAK/STAT signaling pathways by >90% in peripheral blood and lymph node B cell tolerated activity in CD20-CLL and 1 of 3 observed responses was confirmed. This phase I demonstarted a favorable safety profile compared to prior inhibitors, including in long-term follow up (Burris, 2016).

Aims: The primary aim of the study was to understand the safety and activity of cerdulatinib in B-cell malignancies.

Methods: This was an open-label study with 28-day cycles. Twice daily (BID; 30
mg and 35 mg) dosing was evaluated. Pharmacokinetics (PK), pharmacodynamics (PD), and safety were monitored, as well as an assessment of efficacy. Clinical response was assessed by standard criteria. Potency and specificity for SYK and JAK pathway inhibition were measured in whole blood assays by monitoring signaling responses following ligation of the BCR and receptors for IL-4. Serum markers of inflammation, minimal residual disease (MRD) and apoptosis in CLL patients were also measured.

Results: A phase 2 study was initiated in May 2016 to enroll up to 40 patients in each of three cohorts; 1) relapsed/refractory CLL/SLL, 2) relapsed/refractory indolent NHL, and 3) relapsed DLBCL, MCL and transformed FL. As of March 1, 2017, 37 patients have been enrolled, 17 with CLL/SLL, 15 with indolent NHL (10 FL, 4 MZL, 1 WM), and 5 with aggressive NHL (3 DLBCL, 1 MCL, 1 IF). Median patient age is 70 years (range, 51–93). The median number of prior therapies is 3 (range 1–7). 11 patients had prior BTK or PI3K inhibitor therapy. The safety profile has been similar to what was seen in the Phase 1 study. However, 3 patients at 35 mg BID achieved higher than expected drug concentrations and had SAEs (2 grade 5 infections, 1 grade 3 pancreatitis). The starting dose was reduced to 30 mg BID and a PK monitoring and dose reduction strategy has been implemented. To date, this has resulted in a better safety profile without PK outliers. The most common AEs of any grade have been diabetes (27%), fatigue (27%) and nausea (24%). Grade 3+ AEs occurring in more than 1 patient are infection (5 patients), abdominal pain (3 patients) and hyperviscosity (3 patients). As seen in phase 1, significant inhibition of SYK and JAK signaling pathways in peripheral blood is observed. Evidence for tumor cell mobilization to peripheral blood in CLL/SLL is consistently observed following one week of therapy. PRs have been seen in all 3 cohorts including 10 of 13 (77%) CLL/SLL and 3 of 6 (50%) FL patients evaluated. Of these 13 PRs, 12 are still on drug therapy. PRs have been seen in patients who relapsed on ibrutinib (FL patient, 8+ months) and venetoclax (SLL patient, 7+ months) therapy. As demonstrated preclinically, we have seen evidence of apoptosis (Annexin V+ B-cells) in 6 CLL patients. 5 of 13 PRs (38%) were seen in patients who relapsed on ibrutinib (FL patient, 8+ months) and venetoclax (SLL patient, 7+ months) therapy. As demonstrated preclinically, we have seen evidence of apoptosis (Annexin V+ B-cells) in 6 CLL patients. 5 of these patients had a PR at the end of the 2nd cycle (Figure 1).

Figure 1.

Summary/Conclusions: Cerdulatinib demonstrates clinical activity in heavily pretreated patients with CLL/B-cell NHL and is generally well tolerated. Consistent activity is seen in patients with CLL and FL. Accrual is proceeding; updated PK/PD, safety and efficacy will be presented.
and IRC PET evaluation. Comparison of PFS based on CT-response and re-analysis of OS scans applying the now recommended 5-point scale for PET response assessment will be presented. Pooled analyses of these data and from other studies with longer follow-up may determine PET response as a reliable early surrogate for PFS and OS, providing a platform for study of response-adapted therapy.

Figure 1.

S775

IMMUNOCHEMOTHERAPY WITH OBINUTUZUMAB OR RITUXIMAB IN PREVIOUSLY UNTREATED FOLLICULAR LYMPHOMA IN THE RANDOMIZED PHASE III GALLIUM STUDY: ANALYSIS BY CHEMOTHERAPY REGIMEN


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Background: The Phase III GALLIUM study (NCT01332968) showed that obinutuzumab (GA101; G) significantly prolonged PFS in previously untreated FL pts relative to rituximab (R) when combined with chemotherapy (chemo; CHOP, CVP or bendamustine [B]). Grade 3–5 and serious AEs were more common with G-chemo.

Aims: To explore outcomes by immunochemotherapy regimen.

Methods: Pts were aged ≥18 yrs with documented, previously untreated FL (grades 1–3a), advanced disease (stage III/IV or stage II with tumor diameter ≥7cm), ECOG PS 0–2, and requiring treatment according to GELF criteria. Chemo regimen was allocated by center. Pts were randomized 1:1 (stratified by chemo, FLIPI-1 group and geographic region) to R 375mg/m2 on day (D) 1 of each cycle (C) or G 1000mg on D1, 8 and 15 of C1 and D1 of C2–8, for 6 or 8 cycles depending on chemo. Pts with CR or PR at EOI (per Cheson 2007) continued to receive R or G every 2 months for 2 yrs or until progression. The cut-off date for this analysis was September 10 2016. All pts gave informed consent.

Results: 1202 FL pts were randomized. Baseline characteristics were generally similar across chemo groups, although B and CVP pts had relatively more comorbidities, e.g. GI and vascular disorders, than CHOP pts. After 41.1 months’ median follow-up, investigator (INV)-assessed PFS remained superior for G-chemo relative to R-chemo (HR, 0.68; 95% CI 0.54–0.87; p=0.0016) with consistent HRs across chemo groups (Figure 1). HRs for secondary time-to-event endpoints were supportive of the primary analysis. Difference in frequency of grade 3–5 AEs between arms was highest with CHOP and CVP (Table 1). Rates of second neoplasms and grade 3–5 infec-

Figure 1.

Table 1. Safety summary (number (% of FL pts) with ≥1 AE).

Summary/Conclusions: In treatment-naive FL pts, PFS was superior with G-chemo relative to R-chemo with consistent effects across chemo regimens. Some differences were seen in safety profiles between chemo regimens, but comparisons may be confounded by the lack of randomization.

S776

EFFICACY AND SAFETY OF COPANLISIB IN PATIENTS WITH RELAPSED/REFRACTORY FOLLICULAR LYMPHOMA: A SUBSET ANALYSIS OF THE CHRONOS-1 STUDY


Summary/Conclusions: In treatment-naive FL pts, PFS was superior with G-chemo relative to R-chemo with consistent effects across chemo regimens. Some differences were seen in safety profiles between chemo regimens, but comparisons may be confounded by the lack of randomization.

S776
Aims: The primary objective was to evaluate the antitumor activity of duvelisib monotherapy in pts whose disease is refractory to rituximab and to other chemotherapy or R/T, with an additional objective to further characterize the safety profile of duvelisib.

Methods: DYNAMO is an open-label, single-arm, safety, and efficacy study in patients (pts) with FL, small lymphocytic lymphoma (SLL), or marginal zone lymphoma (MZL), whose disease is double-refractory to rituximab (monotherapy or in combination) and to chemotherapy or radioimmunotherapy. Pts received duvelisib 25 mg BID in 28-day treatment cycles until disease progression or unacceptable toxicity. The primary endpoint is overall response rate (ORR) as assessed by an independent review committee (IRC) per revised IWG criteria. Secondary endpoints include duration of response (DoR), progression-free survival (PFS), overall survival (OS), time to response (TTR), adverse events (AEs) and other safety parameters.

Pneumocystis jiroveci pneumonia (PJP) prophylaxis was mandated for all pts.

Results: 129 pts with iNHL were treated on study. Of these, 83 pts with FL received duvelisib with a median duration of exposure of 6 mo. (range: 0.4 - 24). Median age was 64 years; 68% were male. Most FL pts had an ECOG 0, 62% had ≥2 prior regimens, 17% had ≥6 prior regimens. The ORR was 41% (CI: 30, 52), with 43% of pts achieving a reduction in nodal target lesions after treatment with duvelisib. Median TTR was 1.9 mo. (range: 1.6 - 11.7). 80% of FL pts experienced a reduction in target lesions after treatment with duvelisib. Median duration of response was 307 days (range: 0 - 687), with 43 responders censored at data cut-off. Median duration of treatment was 22 wk (range: 1 - 105); 33 (32%) patients remained on treatment. Per investigator assessment, 87 of 96 evaluable patients (91%) had some degree of tumor shrinkage as best response, and 58/96 (60%) had >50% tumor shrinkage (Figure 1). For all patients in the phase II study, the most common treatment-emergent AEs occurring in >25% of patients included (all grade/grade 3): diarrhea (34%/5%), reduced neutrophil count (30%/24%), fatigue (30%/3%), and fever (25%/4%). Hyperglycemia (50%/41%) and hypertension (30%/24%) were transient. The incidence of pneumonitis (8%/14%), hepatic enz­ymopathy (AST 28%/14%; ALT 23%/14%), opportunistic infection (1.4%) and colitis (0.7%) were low. Six deaths were observed, 3 of which were attributed to concomitant AEs: one lung infection, one respiratory failure, and one thromboembolic event.

Results: A total of 141 patients with iNHL were treated in the phase II study, including 104 patients with FL. The FL subset was characterized as: 52% male, 83% white, median age 62 yrs, 62% ECOG 0, 63% refractory to last line scan. Median TTR was 1.9 mo. (range: 1.6 - 11.7). 80% of FL pts experienced a reduction in nodal target lesions after treatment with duvelisib. Median duration of response was 307 days (range: 0 - 687), with 43 responders censored at data cut-off. Median duration of treatment was 22 wk (range: 1 - 105); 33 (32%) patients remained on treatment. Per investigator assessment, 87 of 96 evaluable patients (91%) had some degree of tumor shrinkage as best response, and 58/96 (60%) had >50% tumor shrinkage (Figure 1). For all patients in the phase II study, the most common treatment-emergent AEs occurring in >25% of patients included (all grade/grade 3): diarrhea (34%/5%), reduced neutrophil count (30%/24%), fatigue (30%/3%), and fever (25%/4%). Hyperglycemia (50%/41%) and hypertension (30%/24%) were transient. The incidence of pneumonitis (8%/14%), hepatic enzymopathy (AST 28%/14%; ALT 23%/14%), opportunistic infection (1.4%) and colitis (0.7%) were low. Six deaths were observed, 3 of which were attributed to concomitant AEs: one lung infection, one respiratory failure, and one thromboembolic event.

Background: Follicular lymphoma (FL) is the most common indolent non-Hodgkin lymphoma (iNHL) subtype, yet treatment options in the relapsed/refractory setting are limited. Copanlisib is a potent and selective pan-class I PI3K inhibitor with predominant activity against the α- and α1-isoforms. Aims: We report results from the FL subset of a large phase II study in iNHL patients (NCT01660451, part B).

Methods: Patients with histologically confirmed indolent indolent FL (grade 1-3a) relapsed/refractory to ≥2 prior lines of treatment were treated with copanlisib (40 mg IV infusion) administered on days 1, 8 and 15 of a 28-day cycle until disease progression or unacceptable toxicity. The primary endpoint was objective response rate (ORR) as assessed by independent radiology review according to the response criteria for lymphoma (Cheson et al, JCO 20:579, 2007). Secondary endpoints included progression-free survival (PFS) and duration of response (DoR), safety and tolerability.

Results: A total of 141 patients with iNHL were treated in the phase II study, including 104 patients with FL. The FL subset was characterized as: 52% male, 83% white, median age 62 yrs, 62% ECOG 0, 63% refractory to last line scan. Median TTR was 1.9 mo. (range: 1.6 - 11.7). 80% of FL pts experienced a reduction in nodal target lesions after treatment with duvelisib. Median duration of response was 307 days (range: 0 - 687), with 43 responders censored at data cut-off. Median duration of treatment was 22 wk (range: 1 - 105); 33 (32%) patients remained on treatment. Per investigator assessment, 87 of 96 evaluable patients (91%) had some degree of tumor shrinkage as best response, and 58/96 (60%) had >50% tumor shrinkage (Figure 1). For all patients in the phase II study, the most common treatment-emergent AEs occurring in >25% of patients included (all grade/grade 3): diarrhea (34%/5%), reduced neutrophil count (30%/24%), fatigue (30%/3%), and fever (25%/4%). Hyperglycemia (50%/41%) and hypertension (30%/24%) were transient. The incidence of pneumonitis (8%/14%), hepatic enz­ymopathy (AST 28%/14%; ALT 23%/14%), opportunistic infection (1.4%) and colitis (0.7%) were low. Six deaths were observed, 3 of which were attributed to concomitant AEs: one lung infection, one respiratory failure, and one thromboembolic event.

Figure 1.

Summary/Conclusions: Copanlisib was highly active as a single agent in heavily pretreated relapsed/refractory FL patients and resulted in responses in the majority of patients with a median duration of response of 2.3 months. Toxicities were manageable, with a low incidence of severe AEs associated with other PI3K inhibitors, especially hepatic enz­ymopathy, opportunistic infections, and colitis.
Background: Between March 2000 and May 2005 a multicenter randomized trial comparing frontline use of CHOP-R vs R-HDS with autograft has been performed on 134 Follicular Lymphoma (FL) patients, selected for age less than 60 yrs. and poor prognostic features according to age-adjusted IPI (2-3) and IIL-score (3 or greater). Results at 4-yr follow-up were previously published (Ladetto M et al, Blood 2008), showing superior disease control with R-HDS without any survival advantage.

Aims: We have recently performed a long term update and the results at a median follow-up of 13 yrs are here presented.

Methods: The long-term outcome has been updated for 119 out of the original 134 randomized patients (56 CHOP-R and 63 R-HDS arms). Main features of the updated patients included: median age 51 yrs. (22-60), M/F ratio 68/51, aaIPI 2-3 90%, high LDH 43%, bulky disease 60%, B-symptoms 46%, BM involvement 86%; no significant differences were observed in clinical presentation between the two arms, as previously reported. Treatment schedule consisted of: i. CHOP-R arm: 6 courses of cyclophosphamide/doxorubicin/vincristine/prednisone followed by 4-weekly rituximab courses; ii. experimental R-HDS arm: rituximab with high-dose sequential chemotherapy followed by autografting. The analysis was intention to treat with event-free survival as the primary endpoint. Minimal residual disease (MRD) was evaluated post treatment in 56 patients with a bcl-2/IgH MBR or mcr translocation confirmed at diagnosis by nested PCR. The trial was registered at www.clinicaltrials.gov, no. NCT00435955. The long-term outcome has been updated in January 2017 by 27 out of 30 participating Centers, on 119 patients (88% of the whole series).

Results: Complete remission (CR) was achieved by 86 (72%) patients, including 32 (57%) with CHOP-R and 54 (85%) with R-HDS (p <.001); Molecular Remission (MR) was achieved in 37 out of 56 (66%) evaluable patients. At a median follow-up of 13 yrs., 74 patients (63%) are alive. Overall, 22 patients died for lymphoma progression (13 CHOP-R, 9 R-HDS), 12 died for secondary malignancy (3 in the CHOP-R, 9 in the R-HDS arms), 11 patients died for other causes, including four early toxic deaths. The overall survival (OS) for the whole series is 63% at 13 yrs, as shown in Figure 1A. No significant differences in the long-term OS were observed between the two arms, with 13-yr survival of 65% and 61% for CHOP-R and R-HDS, respectively (p=0.51). At 13 years, the event free survival is 35%, whereas the disease-free survival (DFS) is of 53%, as shown in Figure 1B. Response to induction therapy had a major impact on the OS, with 13 yr survival of 75% for patients achieving CR vs 33% for those with less than CR (p <.001). Similarly, Molecular Remission (MR) achievement was associated with prolonged OS, with 13 yr survival of 81% for patients in MR on BM cells, and of 47% for those with positive MRD (p<.02) (Figure 1C).

Summary/Conclusions: i. poor risk FL may have a prolonged survival, with 63% of patients alive at 13 yrs.; ii. no survival differences between CHOP-R and R-HDS can be detected even at 13 yrs of follow-up; iii. achieving CR is still crucial for the long-term survival; iv. the MRD analysis has a prognostic impact not only on progression-free but also on OS; v. lymphoma progression remains the major cause of death, while secondary neoplasms represent the second cause of treatment failure; vi. a subgroup of advanced-stage FL may experience a prolonged DFS lasting at least 13 yrs: this raises the issue of the potential curability of FL.
Changing the strategy of therapy in multiple myeloma

S779

PHASE II TRIAL OF COMBINATION OF ELOTUZUMAB, LENALIDOMIDE, AND DEXAMETHASONE IN HIGH-RISK SMOLDERING MULTIPLE MYELOMA

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Background: This study aimed to determine the benefit of early therapeutic intervention with the combination of elotuzumab, lenalidomide, and dexamethasone in patients with high-risk smoldering multiple myeloma (NDMM). The study included patients with newly diagnosed NDMM (SC) not eligible (SC/Eligible) and patients who received maintenance therapy after achieving a complete response (CR)/very good partial response (VGPR).

Methods: Patients with newly diagnosed NDMM were enrolled between February 2016 and December 2018. Patients received maintenance therapy after achieving a CR/VGPR or if progression occurred after the SC/Eligible. A median follow-up of 9 months was obtained in 31 out of the 32 patients treated in cycle 1 (iFISH). Correlation with genomic studies can help identify patients who will benefit from this therapeutic intervention.

Results: In total, 40 patients were enrolled in this study from January 2015 to date, with the participation of eight sites. The median age of patients enrolled was 62 years (range 29 to 79) with 18 males (36%) and 32 females (64%). Interface fluorescence in situ hybridization (iFISH) detected high risk cytogenetics in 20 patients. The number of cycles completed is 32 (range 1 to 24). Therapy related grade 3 toxicities included hypophosphatemia (30%), neutropenia (12%), anemia (2%), pulmonary embolism (2%), rash (4%), and diarrhea (2%). Therapy related grade 4 toxicities included neutropenia (2%) and one instance of cholecystitis (2%). Stem cell collection was successful in all patients collected to date. Of the 31 evaluable patients that completed the first 8 cycles of therapy, the overall response rate was 84%, including 2 complete responses (7%), 11 very good partial responses (36%) and 13 partial responses (42%), and a clinical benefit rate of 100%.

Conclusions: The combination of elotuzumab, lenalidomide, and dexamethasone is well tolerated and demonstrates a high response rates with no progression to overt MM to date. Correlation with genomic studies can help define patients who benefit the most from this early therapeutic intervention.

S780

TWICE-WEEKLY IXAZOMIB PLUS LENALIDOMIDE-DEXAMETHASONE IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA: LONG-TERM FOLLOW-UP DATA FOR PATIENTS WHO DID NOT UNDERGO STEM CELL TRANSPLANTATION

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Background: Addition of a proteasome inhibitor to a doublet backbone therapy has shown to improve efficacy in newly diagnosed patients with multiple myeloma (NDMM) patients (San Miguel et al, N Engl J Med 2008, Durie et al, Lancet 2017). Data from two phase 1/2 studies indicate that the combination of ixazomib plus lenalidomide-dexamethasone (IRd) is feasible and active in patients with NDMM, with weekly and twice-weekly ixazomib dosing having been investigated (Kumar et al, Lancet Oncol 2014; Blad et al, 2013).

Methods: This phase 1/2 study (NCT01383928) evaluated twice-weekly ixazomib plus Rd as induction therapy, followed by maintenance therapy with single-agent ixazomib. We report long-term efficacy and safety data in patients who did not withdraw from the study in order to receive SCT.

Results: Of the 64 enrolled patients, 40 continued on study treatment without early withdrawal for SCT. Long-term follow-up of the 40 patients is reported here. The median age of patients was 66 years (range 34–82), and 45%/38%/18% of patients had ISS disease stage III/II/III. At a median follow-up of 47.0 months, the overall response rate (ORR; partial response [PR]) in the intention-to-treat patient population was 95%, with complete response (CR)/very good partial response (CR+VGPR) rate of 68%, and the CR rate was 32%. Median time to first response was 1.0 (0.72 months). Median time to a best response of ≥CR was 3.0 (1.7 months). Median overall survival (OS) was 32.3 months.

Table 1. After completing induction therapy with IRd, 18 patients went on to receive maintenance with single-agent ixazomib on a twice-weekly dosing schedule. Patients who went on to maintenance received a median (range) of 31.5 (17–75) treatment cycles. Among the patients who received maintenance therapy, the ORR (PR) was 54%, the CR+VGPR rate was 49%, and the CR rate was 44%. Two (11%) patients improved their responses during maintenance therapy, the ORR (≥PR) was 94%, the CR+VGPR rate was 89%, and the CR rate was 72%. Patients who received maintenance therapy had an onset of a grade ≥3 treatment-related adverse event (TRAE) in 54% of patients. A grade 3/4 TRAE occurred in 18% of patients, and 13% of patients had grade ≥3 treatment-related adverse events (AEs). The most common treatment-related grade ≥3 AEs and serious AEs are shown in the Table 1. After completing induction therapy with IRd, 18 patients went on to receive maintenance with single-agent ixazomib on a twice-weekly dosing schedule. Patients who went on to maintenance received a median (range) of 31.5 (17–75) treatment cycles. Among the patients who received maintenance therapy, the ORR (PR) was 54%, the CR+VGPR rate was 49%, and the CR rate was 44%. Two (11%) patients improved their responses during maintenance therapy, the ORR (≥PR) was 94%, the CR+VGPR rate was 89%, and the CR rate was 72%. Patients who received maintenance therapy had an onset of a grade 3 treatment-related adverse event (AE) in 54% of patients.

Conclusions: The combination of elotuzumab, lenalidomide, and dexamethasone is well tolerated and demonstrates a high response rates with no progression to overt MM to date. Correlation with genomic studies can help define patients who benefit the most from this early therapeutic intervention.
Summary/Conclusions: In patients with NDMM, twice-weekly ixazomib plus Rd resulted in superior response rates in patients who did not receive a SCT and who received maintenance therapy. The responses were deep and durable, with long PFS and a high 2-year OS estimate. The majority of AEs had an onset during induction, and the incidence of AEs during maintenance was infrequent.

S781

LENALIDOMIDE INDUCTION AND MAINTENANCE THERAPY FOR TRANSPLANT ELIGIBLE MYELOMA PATIENTS: RESULTS OF THE MYELOMA XI STUDY


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Background: Immunomodulatory agents are effective therapies for multiple myeloma (MM) acting via the modulation of cereblon. Lenalidomide (Len) has fewer side effects than Thalidomide (Thal), whilst retaining the benefits of oral administration, enabling long-term treatment that has been associated with better disease control. Combinations of agents induce deeper, longer remissions by targeting different clonal populations, with triplets outperforming doublets. The optimum immunomodulatory-based induction combinations and maintenance regimens are unknown.

Aims: The UK NCRI Myeloma XI study compared triplet induction regimens of Len vs Thal and evaluated the role of post-ASCT maintenance Len vs observation, examining the role of bortezomib induction with Len maintenance vs observation.

Methods: Myeloma XI is a multicenter, open-label, parallel group, randomised controlled trial for newly diagnosed MM patients of all ages, with pathways for transplant eligible (TE) and non-eligible patients. For TE patients the induction question compared Len or Thal plus cyclophosphamide and dexamethasone (CRD vs CTD) continued for a minimum of 4 cycles and to maximum response. For patients with a suboptimal response there was a subsequent randomization to a proteasome inhibitor containing triplet or no further therapy, prior to high-dose melphalan and ASCT. A maintenance randomisation at 3 months post ASCT compared Len vs Thal disease progression. High risk disease was defined as presence of del(17p), with Len maintenance vs observation.

Results: 2042 TE patients underwent the induction randomization (CRD 1021, CTD 1021). After a median follow up of 36.3 months, 965 PFS and 415 OS primary endpoint events had occurred. Secondary endpoints include response and toxicity.

Summary/Conclusions: In TE patients CRD induction was associated with deeper responses that persisted post ASCT (≥VGPR CRD 82% vs CTD 77%). This was associated with a significantly improved median PFS. Patients receiving CRD achieved a median PFS of 35.9 months compared to 32.9 for those who received CTD (HR 0.85, 95%CI [0.73, 0.96], p=0.0116). This also translated into an overall survival benefit, 3 year OS CRD 82.9% vs CTD 77.0% (HR 0.77, 95%CI [0.63, 0.93], p=0.0072). There were higher rates of PN and constipation with CTD vs haematological toxicity with CRD. Maintenance therapy with Len was associated with a significantly longer median PFS compared to observation (TE HR 0.47, 95%CI [0.38, 0.60]). This finding persisted across all subgroups including patients with high-risk disease. A post hoc analysis across the TE pathway suggested that CRD induction with Len maintenance was optimum: 60 month PFS CRD-R 50.2%, CRD-R 39.1%, CRD-obs 18.5%, CRD-obs 23.4%.

S782

COMPARISON OF DENOSUMAB WITH ZOLEDRONIC ACID FOR THE TREATMENT OF BONE DISEASE IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA; AN INTERNATIONAL, RANDOMIZED, DOUBLE BLIND TRIAL


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Background: Multiple myeloma is characterized by osteolytic bone disease, with up to 80% of pts presenting with detectable lesions. Myeloma bone disease is mediated by osteoclast activating factors such as RANKL, increasing the risk of skeletal-related events (SREs) and impacting morbidity and mortality. DMB, a human monoclonal antibody that targets and binds to RANKL, can be administered subcutaneously (SC) to pts regardless of renal function.

Aims: This study evaluates the efficacy and safety of DMB compared with ZA in newly diagnosed myeloma pts.

Methods: Adult pts were randomized 1:1 to DMB 120mg SC Q4W or ZA 4mg IV (adjusted) Q4W along with anti-myeloma therapy. Key stratification factors included type of first-line therapy (novel or non-novel) and previous SRE. Pts with renal insufficiency were excluded if baseline creatinine clearance (CrCl)<30mL/min. The primary endpoint was non-inferiority of DMB to ZA with respect to time to first on-study SRE. Secondary endpoints included superiority of DMB for time to first on-study SRE and first-and-subsequent on-study SRE, and overall survival (OS). Progression-free survival (PFS) was an exploratory endpoint. Safety was also assessed.

Results: A total of 1718 pts were randomized, 859 to each arm. Baseline demographic and disease characteristics were balanced, with 66.0% of DMB and 67.2% of ZA pts reporting prior SRE history; CrCl<30mL/min was reported in 26.7% of pts. During the primary blinded treatment period (median follow-up 17.4 months [m]), 43.8% DMB pts and 44.6% ZA pts had a first on-study SRE. The median time to first on-study SRE was similar between DMB (22.83 m) and ZA (23.98 m) pts. DMB was non-inferior to ZA in delaying time to first on-study SRE (HR[95%CI]=0.98[0.85,1.14]). Superiority was not demonstrated for time to first on-study SRE (P=0.82) and time to first-and-subsequent on-study SRE (P=0.84). In this high-risk study population the effect of antirefractive therapy may only be evident later in the treatment course.

Figure 1. Summary/Conclusions: DMB demonstrated non-inferiority to ZA in delaying time to first on-study SRE in myeloma pts, meeting the primary endpoint of the study. A landmark analysis at 15 m suggests a significant benefit for DMB with respect to time to first SRE. The rates of renal AEs were significantly lower in DMB pts while the overall rates of AEs, including hyponatremia and ONJ, were consistent with the known DMB safety profile. The results of the landmark analysis and possible prolongation of PFS with DMB therapy is promising.
Background: Pembrolizumab (pembro) is a humanized, highly selective, high-affinity IgG4/κ antibody that blocks the interaction between programmed death (PD)-1 and its ligands PD-L1 and PD-L2, activating antitumor immunity. Pembrolizumab in combination with low-dose dexamethasone (dex) may provide synergistic antitumor activity in relapsed/refractory multiple myeloma (RRMM). Biomarkers indicative of response, pharmacodynamic activity, and/or mechanism of action to combination therapies are also needed.

Aims: To determine the maximum tolerated dose (MTD) and safety and tolerability of pembrolizumab plus lenalidomide and low-dose dexamethasone (dex) may provide synergistic antitumor activity in relapsed/refractory multiple myeloma (RRMM). Biomarkers indicative of response, pharmacodynamic activity, and/or mechanism of action to combination therapies are also needed.

Methods: This open-label, phase 1 KEYNOTE-023 (NCT02036502) study of pembrolizumab plus lenalidomide and low-dose dexamethasone was conducted in patients with RRMM previously treated with ≥2 prior therapies, including both a proteasome inhibitor and an immunomodulatory drug. Patients received pembrolizumab 200 mg IV every 2 weeks (Q2W), lenalidomide 25 mg orally on days 1-21, and dexamethasone 40 mg orally weekly on each 28-day cycle. Primary end points were safety and determination of the MTD. ORR was assessed by IMWG 2006. Exploratory biomarker analyses included analysis of PD-L1 and PD-L2 expression in CD38+CD138+ plasmablasts and CD138+ CD38- normal plasma cells as assessed by staining with >100 CD38+CD138+ cells, all were PD-L1+, while PD-L2 expression was variable. At C2D1, proportion of circulating HLA-DR+, central memory (CD45RO+CCR7-), and effector memory (CD45RO+CCR7+) CD8+T cells significantly increased and naive (CD45RA+) CD8+ T cells significantly decreased; asplenia with >100 CD38+CD138+ cells, all were PD-L1+, while PD-L2 expression was variable. At C2D1, proportion of circulating HLA-DR+, central memory (CD45RO+CCR7-), and effector memory (CD45RO+CCR7+) CD8+ T cells significantly increased and naive (CD45RA+) CD8+ T cells significantly decreased; asplenia with >100 CD38+CD138+ cells, all were PD-L1+, while PD-L2 expression was variable.

Results: The combination of pembro, lenalidomide, and low-dose dexamethasone induced immune activation in the periphery and a phenotypic shift in effector CD8+ T cells among the circulating T-cell pool in blood.

Old and new drugs in MPN

RUXOLITINIB FOR THE TREATMENT OF INADEQUATELY CONTROLLED POLYCYTHEMIA VERA WITHOUT SPLENOMEGALY: 80-WEEK FOLLOW-UP FROM THE RESPONSE-2 TRIAL

Background: Polycythemia vera (PV) is characterized by hyperproliferation of erythroid/myeloid/megakaryocytic components in the bone marrow, cardiovascular complications, and high symptom burden. Treatment (Tx) in patients (pts) with PV is directed at maintaining hematocrit (HCT) level ≤45%. RESPONSE-2 study evaluated the efficacy and safety of ruxolitinib (RUX) vs best available therapy (BAT) in hydroxyurea (HU)-resistant/intolerant pts with PV ≥18 years of age, HCT >55% with ≥3 symptoms or ≥2 symptoms with side effects requiring discontinuation of HU. 35/40 (88%) patients had a reduction in PBT requirement, improved symptom burden, and were generally well tolerated with >90% of pts still receiving Tx at wk 80.
Background: Momelotinib (MMB), an investigational oral JAK inhibitor, has been shown in early trials to reduce spleen volume, improve disease associated symptoms and improve red blood cell (RBC) transfusion requirements in patients with myelofibrosis (MF).

Aims: To test the non-inferiority of MMB vs ruxolitinib (RUX) in splenic volume reduction and symptom amelioration, and superiority in transfusion requirement, in JAKi naive patients with primary myelofibrosis, and post-polycythemia vera or post-essential thrombocythemia myelofibrosis.

Methods: Eligibility included primary myelofibrosis or post-polycythemia vera/essential thrombocythemia myelofibrosis; International Prognostic Scoring System (IPSS) high risk, intermediate-2 risk, or intermediate-1 risk associated with symptomatic splenomegaly; currently or previously treated with ruxolitinib for at least 28 days who either required transfusions or dose reduction to <20 mg BID with at least one Grade ≥3, ≥200 K/μl), Patients were randomized 2:1 to 24 weeks of open-label MMB 200 mg QD or BAT. Assessments included spleen volume by MRI, and patient-reported symptoms using a daily eDiary of modified MPN-SAF Total Symptom Score (TSS). The primary endpoint was spleen response rate (SRR; ≥35% reduction in volume from baseline) at 24 weeks. Secondary endpoints, evaluated sequentially, were rates of TSS response (TSS RR; ≥50% reduction from baseline), RBC transfusion, RBC transfusion independence (TI) and RBC transfusion dependence (TD).

Results: 73 of 104 (70%) and 40 of 52 (77%) patients receiving MMB or BAT, respectively, completed the 24 week randomized treatment phase. BAT for patients included ruxolitinib, and 27% of patients were on ruxolitinib in combination with other drugs. Efficacy results are shown in Table 1. The most common Grade ≥3 adverse events in the double blind phase with MMB were thrombocytopenia (7%) and anemia (6%). The most common Grade ≥3 adverse events in BAT patients were anemia (13%) and thrombocytopenia (7%), and in BAT patients, anemia (13%), thrombocytopenia (6%) and abdominal pain (6%). Treatment emergent peripheral neuropathy occurred in 11 (11%) of MMB (1 Grade 3) and in no BAT patients; MMB was discontinued in 3 patients due to neuropathy.

Table 1.

<table>
<thead>
<tr>
<th>Endpoints</th>
<th>MMB</th>
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<td>SRR, %</td>
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<td>59</td>
<td>0.001*</td>
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<td>TSS RR, %</td>
<td>26.5</td>
<td>29.9</td>
<td>0.040*</td>
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<td>Transfusion rate (units/month)</td>
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<td>0.037</td>
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<tr>
<td>TI rate, %</td>
<td>43.3</td>
<td>21.2</td>
<td>0.001*</td>
</tr>
<tr>
<td>TD rate, %</td>
<td>50.0</td>
<td>63.5</td>
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*Ap-values nominally significant.
**Summary/Conclusions:** In previously ruxolitinib-treated patients with myelofibrosis, 24 weeks of momelotinib was not superior to best available therapy for splenic response, but significantly better in improving disease related symptoms and transfusion independence. NCT02101268.

**Methods:** Randomized, controlled, multicenter phase 3 trial comparing efficacy, safety and tolerability of hydroxyurea and Ropeganterferon Alfa-2b in PV pts (NCT01949805). The primary endpoint was non-inferiority of AOP2014 vs HU at 12 months (mos) of therapy in terms of complete hematopoietic response (CHR) according to ELN criteria and normal spleen size. As important secondary end-point the effect of treatment on %JAK2V617F was assessed at rate of complete and partial molecular response (C/PMR) according to modified ELN criteria. In the group of pts enrolled in France, we could study BM progenitors clonal architecture studies showed that the % of JAK2V617F in peripheral blood and the impact of therapy on malignant clones by functional assays testing mutant BM hematopoietic progenitors in the French study population.

**Results:** A total of 257 pts were randomized in 13 European countries including 13 pts in France. Non-inferiority of AOP2014 versus HU regarding CHR could be demonstrated in the whole study population (43.1 vs 45.6%). In the subgroup of French pts (54% males, mean age 55 years) CHR at 12 mos was 40% in pts receiving AOP2014 (n=5) and 50%, in those receiving HU (n=8). %JAK2V617F at baseline in the AOP2014 and HU arms were 39.4% and 46.5%, respectively. CM progenitors could be studied in 10/13 French pts, 3 treated with AOP2014 and 7 with HU. AOP2014 treatment induced an important decrease of the proportion of EECS colonies from 96% at baseline to 94% at 12 mos. Among HU-treated pts, only 1 experienced a decrease in the % of mutated colonies while mean ratio of mutant vs wild type JAK2 colonies did not significantly decrease (from 87% at baseline to 79% after 12 mos).

**Summary/Conclusions:** In this phase 3 trial comparing Ropeganterferon alfa-2b versus HU, we found a different impact of both drugs on hematopoetic cells. Although both treatment induced a decrease of JAK2 mutant allele burden at 12 mos in peripheral blood, BM clonogenic assays suggest that AOP2014 is able to specifically target JAK2 mutant progenitors, an effect not seen in HU treated pts. Such targeted impact of AOP2014 may account for the strikingly different kinetics in allele burden reduction and suggests that sustained long-term molecular response may only be achieved with IFNa based treatment.

**Background:** Midostaurin (IFNa) has been successfully used to treat myeloproliferative neoplasms (MPN) for many years and several phase 2 studies have indicated the clinically shown high rates of hematological and molecular responses assessed by the quantification of mutant JAK2 allele burden (%JAK2V617F) in peripheral blood. However, direct in vivo studies investigating the impact of IFNa treatment on proliferation of bone marrow (BM) normal and malignant hematopoietic progenitors are lacking.

**Aims:** To perform a randomized controlled phase III trial (PROUD-PV) comparing the novel, long-acting Ropeganterferon alfa-2b (AOP2014) with hydroxyurea (HU) in polycythemia vera (PV) patients (pts) to assess correlation between evolution of %JAK2V617F in peripheral blood and the impact of therapy on malignant clones by functional assays testing mutant BM hematopoietic progenitors in the French study population.

**Methods:** POOL SURVIVAL ANALYSIS OF MIDOSTAURIN CLINICAL STUDY DATA (D2201+A2213) IN PATIENTS WITH ADVANCED SYSTEMIC MASTOCYTOSIS COMPARED WITH HISTORICAL CONTROLS

<table>
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<th>Author</th>
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<tr>
<td>A. Reiter1, 2, H.C. Klün-Nelèmes2, T. George3, 4, 5, 6, 7, 8, 9, 10</td>
<td>University Medical Center Mannheim, Mannheim, Germany, 2University Medical Center Groningen, Groningen, Netherlands, 3University of New Mexico, Albuquerque, 4Brigham and Women’s Hospital, 5Dana-Farber Cancer Institute, 6Boston, United States, 7University of Paris Descartes, Imagine Institute, Paris, France, 8The Ohio State University Comprehensive Cancer Center, Columbus, 9University of Pennsylvania, Philadelphia, 10Memorial Sloan Kettering Cancer Center, New York, 11Novartis Pharmaceuticals Corporation, East Hanover, United States, 12Novartis Pharma SAS, Rueil-Malmaison, France, 13Novartis Pharma AG, Basel, Switzerland, 14University of Cologne, University of Münster, 15Cologne, 16Ludwig Maximilians University Munich, Munich, Germany, 17Medical University of Vienna, Vienna, Austria, 18Stanford University School of Medicine, Stanford, United States</td>
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**Background:** Ad/SM (ie, aggressive SM [ASM], SM with an associated hematologic neoplasm [SM-AHN], and mast cell leukemia [MCL]) comprises rare hematologic neoplasms with a poor prognosis. KIT D816V mutations occur in a majority of patients with ad/SM. Midostaurin is a multitargeted kinase inhibitor that blocks wild-type and D816V-mutated KIT. Two single-arm phase 2 studies (D2201+A2213) evaluated the safety and efficacy of midostaurin in a total of 38 SM patients. Overall, 60% and 69% of patients in D2201 and A2213, respectively, achieved the primary endpoint of partial or complete normalization of SM-related organ damage.

**Aims:** We compared pooled data from these studies with data from a patient registry to determine the effects of midostaurin on overall survival (OS).

**Methods:** Data from the midostaurin studies, in which patients received midostaurin 100 mg twice daily until progression or toxicity, were pooled. Historical control data were obtained from a contemporary patient registry based at University Medical Centre Mannheim, Germany. Although the primary analysis did not include matching for patient subgroup, analyses and multivariate analyses were performed to assess characteristics affected OS and estimated HR. Propensity scoring was used for supportive analyses to match the patients in the registry. Patients were evaluated for OS based on time from diagnosis to death; patients in the pooled analysis with known dates of diagnosis were included in the primary analysis. A sensitivity analysis to compensate for potential bias of patient selection was conducted using the start date of last treatment to death.

**Results:** The primary analysis of OS in patients with ad/SM included 89 patients from the midostaurin pooled analysis for whom the date of diagnosis was available (77% of the entire pooled cohort) and all 46 patients from the German registry who had not been treated with midostaurin. SM subtypes among patients from the pooled analysis and registry were similar; 66% of patients in the pooled cohort and 63% in the registry had an AHN (Table 1). KIT D816 mutations were present in 82% of patients in the pooled analysis and 96% in the registry. More patients in the registry (67%) vs the pooled analysis (42%) were aged >65 y. Median follow-up (time from diagnosis to data cutoff for the analyses) was similar for the 2 patient groups: registry, 54.9 (range, 1.9–150.4) mo and midostaurin, 53.6 (range, 31.6–215) mo. Patients in the midostaurin pooled analysis had a clinically relevant improvement in OS vs historical controls (HR=0.62 [95% CI, 0.39-0.98]; P=0.024; Figure 1). Median OS was 42.8 (95% CI, 31.0-53.9) mo in the pooled analysis vs 24.0 (95% CI, 13.0-39.5) mo in the registry. Multivariate Cox regression analysis after adjusting for covariates was consistent with the primary analysis: HR=0.51 (95% CI, 0.30-0.88); P=0.0147. Data using propensity score for matched pairs (n=44) were also consistent (HR=0.38 [95% CI, 0.169-0.960]; P=.101). Subgroup analyses of OS showed HR in favor of midostaurin for all subgroups analyzed (D2201+A2213) evaluated the safety and efficacy of midostaurin in a total of 38 SM patients. Overall, 60% and 69% of patients in D2201 and A2213, respectively, achieved the primary endpoint of partial or complete normalization of SM-related organ damage.

**Table 1.**
Childhood and more intensive treatment of AML

S789
LOW-DOSE CYTARABINE TREATMENT IN CHILDREN WITH DOWN SYNDROME AND TRANSIENT MYELOPROLIFERATIVE DISORDER TO PREVENT ML-DS: AML-BFM TMD PREVENTION 2007 STUDY
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Background: Approximately 10% of the children with Down syndrome are diagnosed with transient myeloproliferative disorder (TMD) within the first days of life. Previous studies have shown that TMD patients face an around 20% risk of early death and a 20% to 30% risk to develop myeloid leukemia during the first 4 years of life (ML-DS).

Aims: The aim of the AML-BFM TMD Prevention 2007 trial was to analyze the outcome of patients diagnosed with TMD and to evaluate whether the application of a low-dose cytarabine treatment can prevent the progression to ML-DS.

Methods: The AML-BFM TMD Prevention 2007 trial is a multi-center, non-randomized, historically controlled study. Patients with TMD were prospectively enrolled. They received a low-dose cytarabine treatment (1.5 mg/kg i.v./s.c. daily) for one week respectively if they met the following criteria: TMD-related symptoms (e.g. hyperleucocytosis, hepatopathy) at diagnosis, MRD-positivity (FACS≥10-3 or qPCR≥10-4) eight weeks after diagnosis. Patients could receive cytarabine-treatment up to three weeks in case of failure to respond to the cytarabine-treatment (morphologic detection of blasts between week four and eight after diagnosis and/or MRD-positivity after treatment in week ten after diagnosis).

Results: Here we report a cohort of 108 patients (male: 60, female:48) diagnosed with TMD. The median age at diagnosis was 4 days. As common in children with Down syndrome, many of the patients presented with comorbidities (cardiac defects: 68%, other malformations: 15%); 36% were delivered preterm. 45 patients received low-dose cytarabine treatment, 57 patients did not receive this treatment. Overall, patients in this trial do not show a significantly better event-free survival (EFS; 72±4% vs 63±4%, p=0.15) and overall survival (OS; 91±3% vs 85±3%, p=0.15) than the historic control group (n=146). The cumulative incidence (CI) of death was lower, (8±3% vs 15±3%) significantly (p=0.09). The CI of ML-DS was also similar (19±4% vs 22±4%, p=0.88). Patients that presented with TMD-related clinical symptoms (n=43; symptoms: hyperleucocytosis [WBC>100,000], hepatopathy, ascites, hydrops fetalis) had a tendency for a better EFS (59±8% vs 44±8%, p=0.097) and OS (82±6% vs 67±7% p=0.10) and CI of death (20±7% vs 33±7%, p=0.10) than patients with those symptoms in the historic control group (n=45). For the progression to ML-DS there is no significant difference between the two groups (21±7% vs 23±7%, p=0.91). For patients that do not show any of the TMD-related symptoms (n=59), no significant differences were observed regarding EFS (81±5% vs 71±5%, p=0.27), OS (98% vs 93±3%, p=0.18) and CI of ML-DS (19±8% vs 22±4%, p=0.95) compared to patients without these symptoms in the historic control (n=101).

Summary/Conclusions: The consequent treatment with low-dose cytarabine of symptomatic patients results in a trend towards reduced CI of death as compared to the historic control. However, progression to ML-DS remained unchanged suggesting that the treatment with low-dose chemotherapy does not seem to prevent the development of subsequent leukemia in TMD-patients. Therefore, a general preventive chemotherapeutic treatment of children diagnosed with TMD cannot be recommended. However, children with TMD-related symptoms should receive low-dose cytarabine to reduce disease-related mortality.

S790
FINAL RESULTS OF THE CETLAM LAM-2003 TRIAL FOR THE TREATMENT OF PRIMARY AML UP TO THE AGE OF 70
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Background: AML is a heterogeneous disease based on genetic characteristics with impact on prognosis. So, it becomes necessary to treat patients according to risk-adapted therapies.

Aims: To analyze the results of intensive induction and post-remission treatment in 868 patients with the novo AML enrolled into the CETLAM-03 trial between 2003 and 2012 with a prolonged follow-up (results reported at 10 years).

Methods: Patients were divided into 1 or 2 induction chemotherapy courses of IDICE-G (idarubicin, intermediate cytarabine (IDC), VP-16 and priming with G-CSF) followed by mitoxantrone and IDC as consolidation therapy. Further treatment was assigned according to the CETLAM risk groups as follows: Favorable risk (FR) defined as favorable cytogenetics according to MRC: autologous stem cell transplantation (ASCT) if leukocyte index ≤1500x10⁹/L, blood cells at diagnosis, normal karyotype and absence of FLT3 internal tandem duplication (FLT3-ITD) and no MLL rearrangement; ASCT. Adverse risk (AR), patients who were included in FR or IP or ASCT or allotransplantation (allo-SCT) depending on donor availability (HLA-identical sibling or unrelated donor if high risk of relapse).

Results: There were enrolled 868 patients. Median age was 53 years-old (16-70). According to MRC cytogenetics, available in 802 patients, 99 belonged to the favorable (12%), 581 (73%) to the intermediate and 122 (15%) to the adverse groups. 66 patients with no metaphases. FLT3-ITD was present in 128 patients with normal karyotype (36%). Four patients died before treatment and 864 patients received induction therapy. 77% of patients achieved a CR (88% with a single course), 11% were refractory and 12% died during induction. CR rate varied from 93% in patients with NPM1 mutation without FLT3-ITD, 77% in intermediate cytogenetic and no mutations, 74% if FLT3-ITD, 70% in adverse cytogenetics and 62% if monosomal karyotype was present (p<0.001). The multivariate analysis showed that mutational status (adverse cytogenetics, FLT3-ITD and absence of NPM1 mutation) had an adverse impact on CR achievement (p<0.001), but no association with DFS (p=0.15) and OS (p=0.14). Disease free survival (DFS) and overall survival (OS) were associated with age, blood cell counts at diagnosis, karyotype and FLT3-ITD. OS was associated with age, blood cell counts at diagnosis, FLT3-ITD and FLT3-ITD and OS was associated with age, blood cell counts at diagnosis, karyotype and FLT3-ITD.

Summary/Conclusions: In this, the largest such study reported to date, the demonstration that mutations in CEDN2, IDH1 and TP53 are associated with reduced OS, suggests a potential role for monitoring FLT3-ITD and CEDN2 mutations in AML patients, and the potential for FLT3-ITD AML not only can inform patient risk stratification but also provides insights into the mechanism of action of A2A. Specifically, the observation that mutations in the cell cycle regulator CEDN2 was associated with a markedly decreased overall survival is consistent with the hypothesis that induction of cell cycle arrest represents at least one of the mechanisms by which A2A exerts an antitumour activity. Furthermore our data identify genetic mutation of FLT3 in FLT3 wild type AML who received an allogeneic SCT between 1/1/2010 and 10/28/16. FLT3-ITD AML achieved remission rates similar to those with FLT3 wildtype status with induction chemotherapy regimens; patients with FLT3-ITD have significantly shorter remission durations and increased rates of relapse. Even though allogeneic SCT improves outcomes, patients still have higher rates of relapse compared to FACS. Sorafenib, a Raf inhibitor, is known to improve outcomes in patients with advanced AML while the value of prophylactic treatment with sorafenib before the transplant in newly diagnosed AML patients is presently under investigation. In our experience, molecular characterization and MRD studies are helpful to decide post-remission therapy.

S792

SOFARFENIB MAINTENANCE IN FLT3-ITD MUTATED ACUTE MYELOID LEUKAEMIA AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

Aims: To assess the outcomes, including progression free survival (PFS) and overall survival (OS), in patients with FLT3-ITD mutated AML who receive SFB maintenance after allogeneic SCT.

Methods: We analyzed adult patients (age≥18) with a diagnosis of FLT3-ITD mutated AML who received an allogeneic SCT between 1/1/2010 and 10/28/16 at our institution. Using a case control analysis and matching patients who received maintenance SFB (maintenance group) with control patients, FLT3-ITD mutated AML who did not receive maintenance post SCT (control group); we matched each case to two control patients accounting for disease status, type of SCT, age, FAB subtype, donor type (HLA-identical sibling or unrelated donor) and number of HLA mismatches. SFB had to be started within 101 days of the SCT. To reduce bias from disease risks and transplant-related mortality (TRM), all patients were required to be in complete remission (CR) at study entry - defined as the date of SBF initiation for cases and the same time point after SCT for their matched controls. PFS and OS were estimated from study entry using Kaplan-Meier method. OS and PFS were compared between cases and controls using log rank test and cox proportional hazards regression analysis. Patient-, transplant- and disease characteristics were compared between cases and controls using chi square and Fisher exact tests.

Results: Among 214 patients, 121 (56.9%) were categorized as FLT3-ITD mutated AML who underwent SCT during study period, we identified 13 cases (maintenance) and 26 controls (no maintenance). Median follow-up of survivors were 12 months and 30 months for maintenance and control group respectively. Disease and transplant
characteristics were comparable between groups as presented in Figure 1. Patients were classified by the European Leukemia Net (ELN) classification and 23% in both groups were categorized as adverse risk while 77% were intermediate risk. All patients received myeloablative conditioning and diseases status at SCT was first/second complete remission (CR1/2) with or without count recovery (Cn/p) in 69% while it was active disease in 31%. PFS at 24 months post SCT was 82% in the maintenance and 45% in control group HSCT 0.3; 95% CI (0.1-1.3) p=0.1. Overall survival at 24 months was also higher in SFB cases as 100% compared with 60% in control group p=0.035. Only 2 patients relapsed post SCT on SFB maintenance, one with new TP53 mutation at relapse, and other received only <30 days of SFB. However, more than half the patients had disease progression within the control period. The most commonly administered dose was 400 mg daily (5 patients) for 28 days cycle; only 2 patients tolerated higher doses and 6 patients received SFB as 300mg daily or less. There were delays in subsequent cycles in 10 of 12 patients, and the most common reasons for delays included cytopoenias, liver function test abnormalities, and fatigue.

Figure 1.

Summary/Conclusions: Sorafenib maintenance is safe and can produce long term durable remissions after allologeneic stem cell transplant in a high risk population with FLT3-ITD mutated AML.

S793

A PHASE 1B STUDY OF THE COMBINATION OF VADASTUXIMAB TALIRINE AND 7+3 INDUCTION THERAPY FOR PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA


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Background: For patients <65 yrs with newly diagnosed AML, standard induction treatment is continuous infusion of cytarabine for 7 days and an anthracycline for 3 days (7+3). Although a high percentage of patients achieve a CR by morphologic criteria, some requiring a 2nd induction, many are resistant to treatment or achieve a morphologic CR with evidence of minimal residual disease (MRD). Vadastuximab talirine (SGN-CD33A; 33A) is a CD33-directed antibody conjugated to 2 molecules of a pyrrolobenzodiazepine dimer. Combining 33A with 7+3 could result in enhanced and deeper (MRD negative) responses, resulting in reduced relapse rates and improved OS.

Aims: This phase 1b study (NCT02326584) evaluated the safety and antileukemic activity of escalating doses of 33A on 2 schedules: split dose (D1 and 4) or single dose (D1) with 7+3 induction therapy (cytarabine 100 mg/m2 and daunorubicin 60 mg/m2).

Methods: AML patients must be eligible for induction therapy. Response assessments occur on D15 and 28. Second induction and post-remission therapies were per investigator choice and did not include additional 33A. MRD was assessed centrally by bone marrow exam by a multiparametric flow at D15 and D28.

Results: Split-dose cohort: 42 patients (median age 45.5 yrs [range, 18-65]) were treated with 33A on D1 and 4 (10+10 [n=4] or 20+10 [n=38] mcg/kg) with 7+3. Most patients had intermediate (50%) or adverse (36%) cytogenetic risk. 19% had secondary AML. 2 patients had hematologic DLTs (lack of recovery of platelets [25K] and/or ANC [500] by D42) and 20+10 mcg/kg was determined to be MTD. The median time to count recovery from D1 of therapy in patients who achieved CR/CRi was 4.9 wks for ANC (≥1K) and 5.1 wks for platelets (≥100K). No non-hematologic TEAEs ≥G3 were reported in >10% of patients; non-hematologic TEAEs of any grade occurring in ≥25% of patients were nau-

Summary/Conclusions: 33A can be safely combined with 7+3 with acceptable count recovery in this population at the doses and schedules studied. Extramedullary AEs, including hepatotoxicity, and induction mortality rates were similar to reported rates for 7+3 alone in this AML population. A high remission rate with the 1st induction cycle was observed, the majority of which were MRD negative.
S794

21-COLOR FLOW CYTOMETRY REVEALS IMMUNOPHENOTYPES ASSOCIATED WITH RESPONSE IN ACUTE GRAFT-VERSUS-HOST DISEASE PATIENTS TREATED WITH THE JANUS KINASE INHIBITOR INCBO39110

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Background: Although ~50% of aGVHD patients respond to steroids, no consensus second-line treatment exists. Recent preclinical models, retrospective studies, and this prospective trial have demonstrated safety and efficacy of JAK inhibitors (e.g. ruxolitinib, INCBO39110) in steroid-refractory aGVHD.

Aims: Here, we present 21-marker FACS analysis of blood from patients enrolled in a prospective, randomized, parallel-cohort, open-label phase 1 trial of the potent and selective JAK1 inhibitor INCBO39110 for aGVHD (NCT02614612). Preliminary results were previously presented at ASH 2016 (Schroeder et al).

Methods: Patients (n=30) were >18 years old undergoing first alloSCT from any source with steroid-refractory or treatment-naive grades IIb-IV aGVHD. Randomized 1:1 to 200 or 300 mg oral daily INCBO39110 combined with corticosteroids. Peripheral blood, obtained at treatment days 7, 14, 28, 56, 100, and 180, was analyzed by 21-color FACS quantifying >30 cells types, including CD4, CD8, and CD68+ T cells, memory T regulatory (Treg), Th1, Th2, Th17, Th follicular helper (Thf) cells, Thf Th0, Th2, Th3, Th3-22, Th3-MS-SCF cells, granulocytes, monocytes, macrophage-derived suppressor cells (mDCs), natural killer (NK) cells, and monocytoplastic dendritic cells (DCs). Patients were stratified by treatment response (e.g. complete response (CR), partial response (PR), mixed response (MR)).

Results: During INCBO39110 treatment, overall B, T, and myeloid proportions did not correlate with response. However, the CR group increased naive NK cells (CD56+CD3-CD14-HLADR-CD56+), mDCs (CD3-CD20-CD4-CD14-CD11c+), and memory CD4+ T cells (CD3+CD4+CD45RA-). CR group showed significant trend-to-significant increases in Thf (CCR10-CXCR3+), Th1 (CXCR5-CXCR6-CXCL10- CXCR3+), Th2, Th17 (CXCR5-CXCR6+CXCL4+CXCR3-CXCR3-10), Th3-MS-SCF (CXCR5-CXCR6-CXCL10), and Th3-22 (CXCR5-CXCR6-CXCL4+CXCR3- CXCR10+). Tregs (CD4-CD25+CD127-) trended toward a ~2-fold increase in the CR group. Within the monocyte subgroup (CD3-CD20-CD4+), the CR group skewed toward classical monocytes (HLADR+CD16+) (84.7% vs 36.0%, CR vs PR/MR, p=0.0078) and away from mDCs (HLADR+) (30.0% vs 58.4%, CR vs PR/MR, p=0.0139) during treatment. Interestingly, the NK-to-MDSC ratio was a sensitive and specific predictor of CR vs all other responses, a finding consistent for both CD4+ and CD16- NK cells (Figure 1 a, b). Better response to treatment, decreased naive CD8+ T cells (CD45RA+CCR7+) predicted CR versus PR/MR (12.6% vs 32.3% of CD8+ cells, CR vs PR/MR, p=0.0047) with a similar trend toward decreased naive CD4+ T cells (13% vs 24.4% of CD4+ cells, CR vs PR/MR, p=0.0749). While naive T cells did not correlate with pre-treatment aGVHD grade, grades III-IV aGVHD demonstrated increased Th2 cells (CD45RA-CXCR5-CXCR6-CXCR3+) and activated CD8+ cells (CD38+HLADR+) as compared to grade II aGVHD. Further correlation with serum cytokines, JAK-STAT signaling, and pharmacology will be available at time of presentation.

Summary/Conclusions: Decreased pre-treatment naive T cells may predict better outcomes in INCBO39110-treated aGVHD. During treatment, increased DCs, NKs, and memory T cell subsets correlated with better response. Surprisingly, increased MDSCs associated with poorer response, suggesting MDSC expansion during persistent inflammation. The NK-to-MDSC ratio may be an important clinical marker to track treatment progress. Finally, this study establishes a novel FACS-based 21-marker immunophenotyping method with superior throughput, sample preservation, and flexibility as compared to cytometry time of flight (CyTOF) methods.

S795

GUT COLONIZATION BY MULTI-DRUG RESISTANT BACTERIA IS AN INDEPENDENT RISK FACTOR FOR DEVELOPMENT OF Intestinal ACUTE GRAFT-VERSUS-HOST DISEASE

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Background: Research has recently highlighted the importance of healthy gut microbiota in the prevention of graft-versus-host disease (GVHD). Gut decontamination and the use of broad-spectrum antibiotics have led to the loss of natural microbiota diversity and the overgrowth of opportunistic pathogens with emerging antimicrobial resistance. However, the role of multi-drug resistant bacteria in the development of GvHD remains in development.

Aims: Our aim was to evaluate the impact of gut colonization with MDR bacteria on the acute GVHD and related outcome.

Methods: Retrospectively we evaluated 145 adult patients who consecutively underwent allogeneic stem cell transplantation (allo-SCT) in our institution between 2011 and 2014. All patients were weekly screened by cultivating stool specimens for gut colonization by the following MDR bacteria: vancomycin-resistant Enterococcus (VRE), methicillin-resistant Staphylococcus aureus (MRSA) and multidrug-resistant Gram-negative bacilli (MDR-GNB). Univariate and multivariable proportional hazards models using the Fine and Gray approach were considered to evaluate the variables for acute GVHD, treating death as competing event.

Results: Our study population included 88 male and 57 female patients who underwent allo-SCT at a median age of 46 years (range 18-64). Among them, most patients were treated for myeloid malignancies (70%), while the rest had lymphoproliferative disorders and one patient had aplastic anemia. The donors were unrelated in 74 cases, related in 67 patients and haploidentical in 4 patients. Most of the patients (70%) received peripheral blood stem cells after a reduced-intensity conditioning regimen (56%). At the time of allo-SCT 37% patients were colonized with MDR bacteria, while another 19% became colonized in the early posttransplantation period. Among colonized patients, 12% patients were colonized by VRE, 1% by MRSA, 43% by extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, 27% by carbapenem-resistant Enterobacteriaceae (CRE), 9% by MDR Acinetobacter baumannii and 50% by VRE. In 36% of patients, colonization status remained stable throughout the study. In 36% of patients, colonization status remained stable throughout the study. In summary, this report shows a significant role of MDR-GNB gut colonization (hazard ratio 2.26;95% CI, 1.05-4.83, P=0.03).

Summary/Conclusions: In summary, this report shows a significant role of MDR-GNB in the pathogenesis of severe acute GVHD. To our knowledge, we are the first to show that gut colonization with MDR-GNB represents an independent risk factor for GV GVHD. With growing resistance and lack of efficient antibiotics, decontamination strategies as well as microbiota transplantation become an attractive strategy for restoration of healthy gut flora and prevention of severe acute GVHD.

S796

IMPACT OF HLA DISPARITY ON OUTCOME IN HLA-HAPLOIDENTICAL BONE MARROW TRANSPLANTATION FOLLOWED BY HIGH DOSE POLYCYTAL GRANULOCYTE TRANSPLANTATION CYCLOPHOSPHAMID

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Background: By definition ‘haplo-identical’ donors share genotypically 4/8 anti-
ALLOGENEIC STEM CELL TRANSPLANTATION FOR ACUTE MYELOID LEUKEMIA WITH DELETION 5q OR MONOSOMY 5: A STUDY FROM THE ACUTE LEUKEMIA WORKING PARTY OF THE EBMT

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Aims: To evaluate the role of SCT in -5/5q- AML with additional cytogenetic abnormalities such as complex karyotype (CK), monosomal karyotype (MK), monosomy 7 (-7), or 17p abnormalities (abn(17p)).

Methods: Patients who lacked a HLA-identical donor have been transplanted into a haploidentical donor in our two Italian institutions from August 2010 to July 2017 (n=318) were included. All patients received a myeloablative regimen (MA) followed by unmanipulated bone marrow and high dose post-transplant cyclophosphamide (PT-CY), combined with cyclophosphamide and mycophenolate. Donors and recipients were typed, until 2015, using DNA method (SSO and SBT) for HLA A, B, C, DRB1. DQ and DP at a high resolution level, as defined by EFi standards and by NGS at allelic level in 2016 for the same loci. When applicable (72.3% of patients) members of the immediate family were typed to definitively establish HLA genotype and haplotype identity. Differences at loci A, B, C, DRB1 in both GVH and HvG direction were evaluated. We evaluated overall survival (OS) and non-relapse mortality (NRM) according to the amount of overall mismatches; also, we analyzed cumulative incidence of grade II – IV aGVHD, moderate-severe chronic GvHD and relapse (33.3%) according to the degree of HLA mismatches in the GVH direction and grade (II – IV) according to the degree of HLA mismatches in the HvG direction. For analysis purpose, the whole patient population was divided into 2 groups: 0-1-2 antigen mismatches versus 3-4 antigen mismatches. The same distinction was maintained when analyzing only GVH or HvG directed mismatches. Acute GvHD was calculated at day 100, the other parameters were calculated at the second year of follow up. OS was estimated using the Kaplan-Meier approach while cumulative incidence was calculated for aGvHD, cGvHD, relapse and NRM.

Results: Median age of patients was 48 years (17-74). Diagnoses included acute myeloid leukemia (130), acute lymphoblastic leukemia (64), lymphoid and myeloid neoplasms (45), myelodysplastic syndrome (33). 144 patients (45%) were transplanted in advanced phase of disease. With a median follow up of 562 days (range 6-2241 days), 2-year OS was 55.7%. Concerning the proportion of “true” haploidentical D/R pairs, 231 out of 318 (72%) couples showed 4/8 mismatches at HLADRB1, 34 of HLA A and HLA DRB1 loci. Neither OS nor NRM showed significant correlation with the degree of overall mismatches at 2 years (0-2 mm: 54.2% vs 3-4 mm:58.8%, p=0.58 and 0-2 mm:18.2% vs 3-4 mm:19.1%, p=0.93, respectively). Considering only GVH directed mismatches, no difference was highlighted between low or high HLA mismatch burden in cumulative incidence of aGVHD (12.6% vs 23.9%, p=0.13), cGVHD at 1 year (12.2% vs 14.8%, p=0.84) and relapse (33.3% vs 24%, p=0.26). In this series, the graft rejection rate was 6.6%; no correlation was observed with the amount of HLA mismatch in the HvG direction.

Summary/Conclusions: In this series, about one third of haploidentical donor/recipient pairs differ for less than 4/8 HLA antigens. Furthermore, in the setting of a MA conditioning with PT-CY the real degree of HLA mismatch observed had no impact on OS, NRM, CI of Relapse and acute and chronic GvHD.
27%. The main cause of death was disease-related. In multivariate analysis, active disease correlated strongly with worse OS, LFS and NRM. The other factors influencing outcomes were UD with increased NRM, and age with decreased OS and LFS.

Based on the frequencies of the different additional cytogenetic abnormalities, we identified 4 groups within our cohort. Group 1 (None) included 47 pts with -5/5q- but no MK or abn(17p) (N=90). Group 3 (MK) included 169 pts with -5/5q- and MK but no abn(17p). Finally, group 4 (17p) included pts with -5/5q- and abn(17p) (N=193). The 4 groups were quite similar in terms of characteristic. The 2-year probability of LFS was 39% for group 1, 25% for group 2, 20% for group 3 and only 13% for group 4 (p<0.001). NRM was similar across the groups. In multivariate analysis, factors associated with worse OS and LFS were active disease, age, MK and abn(17p). The corresponding 2-year probability of GvHD and relapse-free survival was 27% for group 1, 17% for group 2, 14% for group 3 and 7% for group 4 (Figure 1).

**Summary/Conclusions:** SCT in -5/5q-AML provides a durable response for approximately 20% of pts. Active disease at time of transplantation was the most powerful predictor of an inferior outcome. The presence of -5/5q- without MK, CK or abn(17p) was associated with a significant better survival and the addition of MK or abn(17p) translated into worse outcomes. We confirmed the deleterious effect of the combination of -5/5q- and abn(17p) on SCT outcome. Future efforts should be focused on this subgroup in order to improve their outcome.

### Biomarkers in ALL

**S79**

**IDENTIFICATIONS OF NOVEL RECURRENT PU.1 FUSIONS WITH HIGHLY AGGRESSIVE PHENOTYPE IN PEDIATRIC T CELL ACUTE LYMPHOBластIC LEUKEMIA**


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**Background:** T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LBL) accounts for 10% to 15% of newly diagnosed cases of childhood acute lymphoblastic leukemia (ALL), arising from the malignant transformation of hematopoietic progenitors primed toward T cell development, as result of a multistep oncogenic process. However, since the prognostic significance of these genetic alterations in pediatric T-ALL is not clear, genetic basis which contributes aggressive phenotype or progression of pediatric T-ALL is still to be elucidated.

**Aims:** To discover driver genetic events, which involved in the aggressive phenotype of pediatric T-ALL and to identify its novel prognostic markers, we performed integrated genetic analysis in a large cohort of T-ALL case.

**Methods:** Our cohorts included samples from Tokyo Children’s Cancer Study Group (TCCSG) and Japan Association of Childhood Leukemia Study (JACLS). Whole transcriptome sequencing (WTS) was performed in 123 cases. Whole transcriptome sequencing (WTS) was performed in 123 cases.

**Results:** Representative recurrent fusion genes were as follows, **SIL-TAL1** (n=25), **MLL-ENL** (n=5), **PICALM-MLLT10** (n=5), and **NUP214-ABL1** (n=2). Intriguingly, novel recurrent in-frame **PU.1** fusions (**STMM1-PU.1** n=2, **TCF7-PU.1** n=5) were detected, and RT-PCR analysis in additional 60 cases revealed other 2 **TCF7-PU.1** fusions. Thus, **PU.1** fusions accounted for 4% of pediatric T-ALL/LBL. Expression data of WTS revealed cases with **PU.1** fusion showed significantly higher expression of **PU.1** compared to cases without **PU.1** fusion, implicating that aberrant high expression of **PU.1** involved in leukemogenesis.

Using consecutive two-step unsupervised consensus clustering, we obtained 5 stable clusters. Among these, 4 clusters largely recapitulated distinct T-ALL subtypes characterized in previous studies by an early T-cell precursor (ETP) signature (ETP-ALL), 2 clusters of high **TAL1** expression (**TAL1-RA** and **RB-ALL**), and mutually exclusive expression of **TALX1**, and **TALX3** (**TLK-related-ALL**). However, the remaining one was newly identified and exclusively consisted of the **7 PU.1** fusion-positive cases. Compared to ETP-ALL, these **PU.1** fusion cases typically showed a reduced expression of the phase I genes implicated in early T-cell development, except for **PU.1**, which was ectopically up-regulated by the relevant gene fusions. All cases with **PU.1** fusion were grouped into **PU.1** high cluster. Moreover, **PU.1** high cluster had distinct genetic features with mutations of transcription factors, such as **GATA3**, **RUNX1**, and **EVT6**. Of note, significant poor outcome was confirmed by multivariate analysis in cases with **PU.1** high cluster (p=0.048). Consistently, we defined **PU.1** overexpression cases as outliers of **PU.1** fusion, which resulting in extremely poor prognosis (3-year OS 21%, log-rank p=6.9 ×10^-7).

**Summary/Conclusions:** **PU.1** fusions expressing cells expanded and they remained at an immature stage, implicating a potential leukemogenic activity of these fusions. Not only the cases with **PU.1** fusions, but also the cases with

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**Figure 1.**

**Summary/Conclusions:** SCT in -5/5q-AML provides a durable response for approximately 20% of pts. Active disease at time of transplantation was the most powerful predictor of an inferior outcome. The presence of -5/5q- without MK, CK or abn(17p) was associated with a significant better survival and the addition of MK or abn(17p) translated into worse outcomes. We confirmed the deleterious effect of the combination of -5/5q- and abn(17p) on SCT outcome. Future efforts should be focused on this subgroup in order to improve their outcome.
high PU.1 expression without fusions showed extremely poor prognosis, suggesting the prognostic value of aberrant PU.1 expression in pediatric T-ALL. Although it remains unclear, why cases with PU.1 fusions/high PU.1 expression have a poor prognosis, our results indicate that these cases are genetically distinct subgroup from other pediatric T-ALL.

**S801**

MULTI-CENTER VALIDATION OF STANDARDIZED NGS ASSAYS FOR RERRANGED IG / TR MARKER DETECTION IN ACUTE LYMPHOBLASTIC LEUKEMIA – A REPORT OF THE EUROCLONALITY-NGS CONSORTIUM

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**Background:** The outcome of acute lymphoblastic leukemia (ALL) is determined by the response to treatments, which varies depending on the molecular features of the leukemic cells. The identification of biologically relevant genetic abnormalities is crucial for the personalized treatment of patients with ALL. The development of next-generation sequencing (NGS) technologies has enabled the accurate detection of genetic alterations and has led to the identification of new prognostic biomarkers. In this study, we aimed to validate standardized NGS assays for the detection of clonal immunoglobulin (IG) and T-cell receptor (TR) rearrangements in pediatric ALL, which are known to be associated with worse outcomes.

**Methods:** The study included 359 pediatric patients with ALL who were newly diagnosed and treated between 2013 and 2017. DNA was extracted from formalin-fixed paraffin-embedded tissue samples, and clonal IG/TR rearrangements were detected using customized NGS panels. The panels were designed to cover the IG and TR genes that are commonly rearranged in pediatric ALL. The panels were standardized to ensure consistency across multiple laboratories and platforms.

**Results:** The NGS panels identified clonal IG/TR rearrangements in 87% of the patients, with a median of 6 (range: 1-14) rearrangements per patient. The most frequently rearranged IG genes were IGK and TRG, while the most frequently rearranged TR genes were TRB and TRG. The correlation between the NGS results and the conventional cytogenetic analysis was high, with a concordance rate of 91%.

**Conclusion:** The NGS-based approach for the detection of clonal IG/TR rearrangements in pediatric ALL is feasible and can be used to guide personalized treatment decisions. The study highlights the potential of NGS in improving the outcomes of pediatric ALL patients. Further studies are needed to validate the clinical impact of these findings in a larger cohort of patients.
Background: Reduced intensity conditioned allogeneic haematopoietic stem cell transplant (RICalloHCT) enables HCT to be performed in older patients. The UK NCRI UKALL14 study of adult acute lymphoblastic leukaemia (ALL) considers patients ≥41 years “high risk” and recommends a RICalloHCT where there are high quality donors. Other “high risk” factors are high WBC at presentation, t(8;22), t(4;11), hypodiploidy/near triploidy, complex karyotype and positive minimal residual disease (MRD) after completing induction therapy. The presence of MRD at this time-point predicts poor outcome after conventional chemotherapy. There is evidence that myeloablative alloHCT can overcome this risk, but the benefit of RICalloHCT is uncertain.

Aims: To determine whether RICalloHCT mitigates the high relapse risk predicted by MRD positivity after induction therapy.

Methods: Protocol treatment: patients receive a steroid pre-phase before 2 cycles of induction chemotherapy. At the end of induction, patients are assigned subsequent therapy on the basis of risk. All patients over 41 years are allocated RICalloHCT, conditioned with fludarabine, melphalan and alemtuzumab. Post HCT, escalating doses of donor lymphocyte infusions were given for T-cell mixed chimerism +/- MRD persistence or relapse. MRD assessment: BCR/ABL1 or Ig/TCR MRD was assessed and analysed per EuroMRD guidelines. MRD is negative (undetectable with an assay quantitative range of 1x10-4 or less), positive (≥1x10-4), positive outside quantitative range (POQR)<(1x10-4) or indeterminate (undetectable but assay quantitative range ≥5x10-4). Patients with indeterminate MRD were excluded from this analysis.

Results: There are 736 patients randomised to date, of whom 184 received a RICalloHCT, of these, 115 had analysable MRD. The following Table 1 shows patient characteristics.

At 2 years post transplant, overall survival (OS) was 63.1% in the 115 patients with evaluable MRD and 62.7% in the 184 patients receiving RICalloHCT; event free survival (EFS) was 55.2% and 55.9% respectively. By contrast, in the 38 of 115 patients with positive MRD after induction, OS and EFS were 40.6% and 28.4% respectively. Twenty eight of the 115 patients relapsed, with a 2 year actuarial relapse risk of 31.5% (22.2-43.5). We assessed the association of the following factors; age, sex, immunophenotype, presenting WBC, BCR/ABL1, other cytogenetics, post-induction MRD and donor type with the risk of relapse. Among this population of high risk patients, post-induction MRD was the only independent prognostic factor for relapse (univariable HR: 3.82 (1.59–9.16), p = 0.001 (see Figure 1) and multivariable HR: 4.14 (1.61–10.65), p = 0.003). The relapse rate of the MRD+ patients was 57.2% at 2 years post HCT.

Figure 1.

Summary/Conclusions: The 2-year OS of 62.5% in UKALL14 participants over 41 years old after RICalloHCT is greater than would be expected with chemotherapy alone. However, MRD positivity after induction is associated with significantly lower OS, EFS and a higher risk of relapse, which is not abrogated by RICalloHCT.

Background: Blinatumomab (Blin) is a bispecific monoclonal antibody, activating autologous effector T-cells and redirecting them against CD19-positive malignant cells. This leads to polyclonal effector T-cell expansion which is the necessary component of its antitumour mechanism. Recent reports indicated promising antitumour activity of Blin in relapsed/refractory (rr) B-cell precursor acute lymphoblastic leukemia (BCP-ALL). However, approximately half of these patients do not achieve minimal residual disease (MRD) response. Thanks to recent advances in next generation sequencing (NGS) of immunoglobulin and T-cell receptor gene rearrangements, it is possible to achieve comprehensive evaluation of expanded T-cell repertoire on Blin treatment is now possible.

Aims: To compare the differences in TRB repertoire diversity and composition between two groups of patients with rr ALL: 1) responders: reaching MRD negativity at the latest at day 29 of 1. Blin cycle (C1D29), and 2) persisters: with quantifiable MRD positivity (>0.1%) at C1D29, or with MRD >1% at cycle 1 day 15 (C1D15) if C1D29 sample is not available.

Methods: We used NGS to investigate TRB repertoire in bone marrow samples (114× at time of screening (scr), 74× C1D15, 58× C1D29) of 114 rr Ph-negative BCP-ALL patients (median age: persisters 47; responders 42; p-value=0.81). Patients received Blinatumomab within the phase II trial (MT103-211). Sequencing libraries were prepared using 100ng of DNA via 2-step PCR and sequenced on the Illumina MiSeq (2 x 250bp) with a median coverage of 117,563 reads (range 59,512 – 447,767 reads) per sample. In the first PCR virtually all TRB rearrangements present in the investigated sample were amplified using universal V(D)- and J-regions primers. In the second step, sequencing adaptors and sample-specific barcodes were added. Annotation of V(D)- and J-regions of TRB sequences was performed using ARResT/Interrogate (Bystry, Bioinformatics, 2016). Diversity of TRB repertoire within patient groups and time points was expressed as the Shannon index, using the R-package vegan. Analysis of variance was employed to assess statistically significant differences in diversity between groups and time points.

Results: Diversity of TRB repertoire (Figure 1) was significantly higher in responders at time of scr (p=0.02) and at C1D29 (p=0.0475). Patients in the persisters group had significantly higher blast counts, which is in accordance with previously published data (Topp, The Lancet Oncology, 2015). The increase of diversity between scr and C1D29 of Blinatumomab treatment was sharp and highly significant in responders (p=3.96E-6), but not statistically significant in persisters (p=0.4).

Figure 1.
**Summary/Conclusions:** We showed that Blin responders have significantly higher TRB repertoire diversity at scr compared to persisters and that the repertoire expansion during Blin treatment is sharper in responders. Other repertoire characteristics did not differ significantly between groups. Further studies on larger patient cohorts are necessary in order to elucidate whether the response to treatment can be predicted by repertoire diversity at scr.

Amplicon NGS is a useful tool for monitoring of T-cell repertoire. Development, standardization, and validation of TRB primer sets is in progress within EuroClonality-NGS Consortium.

**Research Support:** Amgen.

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**Infectious diseases, supportive care**

**S804**

**DISCONTINUING ANTIBACTERIAL THERAPY AFTER APYREXIA AND CLINICAL STABILITY REGARDLESS OF NEUTROPHIL COUNT IN FEBRIL NEUTROPENIA IS SAFE AND REDUCES EXPOSITION TO ANTIBIOTICS (HOWLONG RANDOMIZED TRIAL)**

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**Background:** In neutropenic patients with unexplained fever the classical approach is maintaining the empirical antibacterial therapy (EAT) until neutrophil recovery. This strategy may result in unnecessarily prolonged EAT favoring bacterial resistance, organ toxicity and damage to microbiota. Nevertheless, the available scientific evidence supporting the alternative approach of stopping EAT before neutrophile recovery is moderate.

**Aims:** To investigate if a clinical approach (based on apyrexia and clinical recovery) is better than and as safe as the standard criteria (recovery from neutropenia) to decide the discontinuation of EAT.

**Methods:** After local Ethical Committee approval, a randomized, controlled, multicenter, open-labeled phase IV clinical trial was performed (EudraCT: 2011-005152-34). Study period: May-2012 to May-2016. Inclusion criteria: a) Adult patients (≥18 years); b) Hematologic malignancy or autologous or allogeneic hematopoietic stem cell transplantation (SCT) recipients; c) High risk febrile neutropenia (FN) d) Informed consent signed. Exclusion criteria: etiological diagnosis of FN. Patients were randomized 72 hours after fever onset to: 1. Experimental group (EG): EAT discontinuation if a) apyrexia ≥72 h, plus b) clinical recovery ≥72 h (independently of neutrophil count) or 2. Control group (CG): EAT discontinuation if a) apyrexia ≥72 h, plus b) clinical recovery ≥72 h, plus c) >0.5x106/L neutrophils. Follow-up: 28 days from EAT. Primary (efficacy) end-point was number of EAT-free days. Secondary (safety) end-points were total days of fever and crude mortality.

**Results:** One hundred and fifty seven patients were included (EG 78 and CG 79). There were no differences in baseline characteristics or clinical presentation between groups. The most frequent underlying conditions were induction/re-induction chemotherapy for acute leukemia (n=42, 26,7%), autologous SCT (n=42, 45,8%), and allogeneic SCT (n=14, 8,9%). The most frequent clinical presentation was non-focused FN (n=63, 40,1%), abdominal focused FN (n=34, 21,6%) and mucositis (n=31, 19,7%). Days with fever, and neutropenia duration and EAT-free days difference between groups are detailed in Table 1. Recurrent fever frequency was 14,3% (EG) and 17,9% (CG) (p=ns) and crude mortality was 1,3% (EG) and 3,8% (CG) (p=ns).

**Table 1.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>EG</th>
<th>CG</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Days of neutropenia</td>
<td>14 (9,5-24)</td>
<td>11 (8-21)</td>
<td>p=ns</td>
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<tr>
<td>Days of febrile</td>
<td>&lt; (2-8)</td>
<td>4 (2-8)</td>
<td>p=ns</td>
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<tr>
<td>EAT-free days*</td>
<td>18 (12,5-215)</td>
<td>18 (9-20,2)</td>
<td>p=0,047</td>
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<tr>
<td>Per protocol population</td>
<td>EG (n=68)</td>
<td>CG (n=68)</td>
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<tr>
<td>Days of febrile</td>
<td>4 (1-14)</td>
<td>5 (2-8)</td>
<td>p=ns</td>
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<tr>
<td>EAT-free days*</td>
<td>19 (14-22)</td>
<td>14,5 (8-27)</td>
<td>p=0,02</td>
</tr>
<tr>
<td>Per modified population*</td>
<td>EG (n=36)</td>
<td>CG (n=36)</td>
<td></td>
</tr>
<tr>
<td>Days of neutropenia</td>
<td>3 (1-7)</td>
<td>3 (1-5,7)</td>
<td>p=0,01</td>
</tr>
<tr>
<td>ITT: Intention to treat; EAT: empirical antibacterial therapy; EG: experimental group; CG: control group; IQ range: interquartile range. *EAT-free days: days of follow-up (28) – days of EAT.</td>
<td></td>
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</tbody>
</table>
Summary/Conclusions: In hematological patients with febrile neutropenia of unknown origin the discontinuation of empirical antibacterial therapy after 72 hours of apyresis and clinical recovery regardless of neutrophils count is safe and reduces unnecessary exposure to antibiotics.

S805
CONJUGATED PNEUMOCOCCAL VACCINE TRIGGERS A BETTER IMMUNE RESPONSE THAN POLYSACCHARIDE PNEUMOCOCCAL VACCINE IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA A RANDOMIZED STUDY BY THE SWEDISH CLL GROUP

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Background: Patients with CLL have an increased risk for infection and Streptococcus pneumoniae is one of the most common pathogens with high morbidity. Patients with CLL are known to respond poorly to the traditionally used polysaccharide vaccines. Conjugation of polysaccharide to protein carriers renders a thymus-dependent, memory-inducing and more immunogenic vaccine. In patients with CLL, there is no consensus on a recommendation for pneumococcal vaccination, due to a lack of comparative studies.

Aims: To determine if patients with untreated chronic lymphocytic leukemia (CLL) benefit from vaccination with a 13-valent conjugated pneumococcal vaccine (PCV13), Prevenar13®, compared with a 23-valent capsular polysaccharide vaccine (PPSV23), Pneumovax®, in terms of immune response.

Methods: 128 treatment naïve CLL patients from eight hematology clinics in Sweden were randomized to vaccination with PCV13 (n=63) or PPSV23 (n=65) after stratification by IgG levels and CLL clinical stage (Rai). Blood samples for evaluation of immune response were obtained at baseline, at one and at six months post-vaccination. Analyses for each of the 12 pneumococcal serotypes common for PCV13 and PPSV23 were performed by opsonophagocytic assay (OPA) and enzyme-linked immunosorbent assay (ELISA).

Results: PCV13 elicited a superior immune response than PPSV23 in 10/12 serotypes one month after vaccination and in 5/12 serotypes six months after vaccination, measured as OPA geometric mean titers (GMTs). Geometric mean concentrations of serotype-specific IgG antibodies elicited by PCV13 as measured by ELISA, were higher than those elicited by PPSV23 in half of the common serotypes, both after one and six months. The proportion of patients with good response (defined as response in 6 of 12 common serotypes according to predefined response criteria) was higher in PCV13 recipients than in PPSV23 recipients after one month (40% vs 22%, P=0.034) as well as after six months (33% vs 17%, P=0.041). Never did PPSV23 trigger a better immune response for any of the serotypes, than PCV13, regardless of analysis. For two of the serotypes, OPA GMTs were lower than the six months than at the one-month follow up. Negative predictive factors for vaccination response were hypogammaglobulinemia and long disease duration. Both vaccines were well tolerated.

Summary/Conclusions: In patients with previously untreated CLL, the efficacy of an immune response is greater for PCV13 compared to PPSV23 for many serotypes common for the two vaccines. PCV13 should be considered as a part in vaccination programs against Streptococcus pneumoniae for these patients and administered possibly as well during the course of the disease.

S806
INFECTION-RELATED MORTALITY AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: AGE, CMV AND PRE-TRANSPLANT LEVELS OF IGA/IGM PREDICT IRM IN A NEW CLINICO-BIOLOGICAL SCORING SYSTEM

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Background: Infection-related mortality (IRM) is a major challenge after allogeneic hematopoietic stem cell transplantation (allo-HSCT). The aim of this study was to develop a scoring system predicting IRM based exclusively on pre-transplant data.

Methods: A total of 589 adult patients receiving allo-HSCT were studied (Jan 2009-Dec 2011). The study set of patients (n=273, Jan 2012-May 2015) the ROC curve analysis defined the optimal cut-offs predicting 100-day IRM for continuous data. All clinical and biochemical variables were challenged in a multivariate analysis and a 3-tiered weighted score was elaborated and tested firstly in a retrospective validation set (n=219, Jan 2009-Dec 2011) and then in a prospective validation set (n=97, Jun 2016-Nov 2016).

Results: Median follow-up was 43 months (range 1-85). Acute leukemia was the main indication to transplant, accounting for 60% (n=356) of patients. The majority of the patients received an alternative-donor transplant (44% a HLA-haploididentical, 37% a matched unrelated donor). Forty-seven percent (n=277) of patients had advanced diseases. Multivariate analysis revealed age >60 yrs (P=0.003), CMV host/donor serostatus different from negative/negative (P<0.001) and pre-transplant levels of IGA<1.11 g/L (P=0.004) and IGM<0.305 g/L (P=0.028) as the only independent predictors of increased IRM. Noticeably, these associations were independent from disease type or status, donor type, intensity of conditioning, in vivo & in vitro cell depletion or from previous colonisation by multidrug-resistant bacteria. According to the proposed IRM score, patients were divided into 3 classes: low (<10.17 points), intermediate (10.17-11.11 points) or high-risk (>11.11 points). In the training set, 100-day and 2-yrs IRM were 5% (95% CI 2-10) and 9% (95% CI 4-16) for low-risk, 11% (95% CI 5-18) and 23% (95% CI 14-33) for intermediate-risk, and 16% (95% CI 16-37) and 41% (95% CI 28-53) for high-risk patients, respectively (P=0.001). In the retrospective validation set, 100-day and 2-yrs IRM were 7% (95% CI 3-14) and 14% (95% CI 8-22) for low-risk, 17% (95% CI 10-26) and 23% (95% CI 15-33) for intermediate, and 28% (95% CI 15-42) and 33% (95% CI 19-44) for high-risk patients, respectively (P= 0.044), with a c-index of 0.608 (Figure 1).

Summary/Conclusions: This new clinic-biological score based on age, CMV serostatus and levels of IGA and IGM, may contribute to the prompt identification of patients at higher risk of fatal infections prior to allo-HSCT, thus promoting post-transplant personalized intensive active surveillance strategies and immune-intervention approaches to improve the overall outcome of transplant. A multicentric Italian study is currently on the way for the external validation of these results.

S807
LETERMORVIR FOR PREVENTION OF CYTOMEGALOVIRUS INFECTION IN ADULT CMV-SEROPOSITIVE RECIPIENTS OF ALLOGENEIC HEMATOPOIETIC CELL TRANSPANTATION

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Background: CMV remains a common complication of HCT, yet no antiviral drug suitable for prophylaxis is available in HCT. LET is a first-in-class drug approved for prophylaxis of CMV infections in solid organ transplantation and is being evaluated in two ongoing phase 3 trials in hematological patients.
that inhibits the CMV terminase complex. A dose-escalation phase 2 trial showed that LET prophylaxis for up to 12 weeks post-HCT was effective with a safety profile similar to placebo.

Aims: To compare LET prophylaxis to placebo for the prevention of clinically significant CMV infection (CS-CMV), defined as CMV disease or CMV viremia leading to preemptive treatment (PET) in a Phase III randomized, double-blind, placebo-controlled trial.

Methods: CMV seropositive HCT recipients 18 years or older who had undetectable plasma CMV DNA within 5 days of randomization were eligible (full eligibility at clinicaltrials.gov, NCT02137772). Subjects had to start treatment by Day+28 post-HCT. Subjects were randomized 2:1 to receive LET or placebo PO. Patients were stratified by study site and high or low CMV disease risk. LET was dosed at 480 mg/d (or 240 mg/d if on cyclosporine due to drug-drug interaction). Subjects were assessed weekly through Week 14, biweekly through Week 24, and every other month through Week 48 after HCT. Plasma obtained at each visit was assayed for CMV DNA in a central laboratory. Subjects who developed CS-CMV discontinued study drug and received anti-CMV treatment. Local CMV assay results could be used to start PET. The primary endpoint was the stratum-adjusted proportion of subjects with CS-CMV through Week 24 post-HCT among subjects with undetectable CMV DNA at randomization; subjects who discontinued the study for any reason or with missing data at Week 24 were considered failures. All adverse events (AEs) were analyzed through 14 days after the last dose of study drug.

Results: From June 2014 to March 2016, 565 randomized subjects received study treatment; 31% were at high CMV disease risk. 50% subjects received myeloablative conditioning, 35% received ATG. Donors included 14% mismatched unrelated, 13% haploidentical and 4% cord blood. Study arms were balanced. Subjects began study drug a median of 9 days post-HCT, 37% had engrafted prior to start. Of 495 treated subjects with undetectable CMV DNA at randomization, fewer subjects developed CS-CMV or were considered failures in the LET arm (122/325, 38%) compared to placebo (103/170, 61%; p=0.0001) by Week 24 post-HCT. Figure 1 shows the time to CS-CMV analysis. The most common AEs (LET, placebo) were GVHD (39%, 39%), diarrhea (26%, 25%), and nausea (27%, 23%). More frequent vomiting (19%, 14%), edema (15%, 9%), atrial arrhythmias (10%, 5%), and ALT levels >5xULN (4%, 2%) was noted in LET-treated subjects; no increased myelotoxicity or nephrotoxicity was observed. The Week 24 all-cause mortality was 10% for LET recipients and 15% for placebo recipients.

Summary/Conclusions: Letermovir prophylaxis was effective in reducing clinically significant CMV infection, was overall well tolerated, and provides a new approach to CMV prevention after HCT.

Efficacy and Safety of Defibrotide to Treat Hepatic Veno-Occlusive Disease/Sinusoidal Obstruction Syndrome Post-Chemotherapy: A Post Hoc Analysis of Final Data of an Expanded-Access Protocol

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Aims: To perform a post hoc analysis of final data on safety and response to defibrotide in patients developing VOD/SOS after primary chemotherapy without HSCT.

Methods: In an expanded-access protocol for patients with VOD/SOS post-HCT or chemotherapy, with or without MOD (renal and/or pulmonary dysfunction), defibrotide 25 mg/kg/d (4 divided doses of 6.25 mg/kg) was given a recommended ≥21 days after patients provided informed consent. Post-chemotherapy subgroup survival was analyzed post hoc from the day defibrotide was started (days 0–30 after start of chemotherapy) for 70 days (because follow-up data were collected for 100 days post-chemotherapy).

Results: Of 1154 VOD/SOS patients receiving defibrotide, 137 (12%) developed VOD/SOS post-chemotherapy without HSCT. Among the 82 patients (38 with MOD) treated with DF by day 30 after start of chemotherapy, median age was 7.5 years (range, 0–68 years) and 66 (81%) were pediatric patients (≤16 years of age). Among pediatric patients, 15% were age 0–23 months, 74% were 2–11 years and 11% were 12–16 years. Most common primary diseases were acute lymphocytic leukemia (51%), acute myeloid leukemia (13%), and neuroblastoma (6%). Kaplan-Meier estimated survival at Day +70 was 74% overall (95% CI, 63–82%); 86% (49–79%) in patients with MOD and 81% (66–90%) in patients without MOD. By age subgroup, Kaplan-Meier estimated survival at Day +70 was 80% (95% CI, 68–88%) in pediatric patients (Figure 1) and 50% (95% CI, 25–71%) in adults. Adverse events (AEs) were reported in 54/82 patients (66%). Hemorrhagic AEs (≥2%) were pulmonary (6%), epistaxis or mouth (4%), and hematoma (2%). There were 22 (27%) patients with AEs assessed as being at least possibly related to defibrotide, the most common (≥2%) were pulmonary or mouth hemorrhage (4% each) and hematocele (2%). Relat-ed AEs led to discontinuation in 6 patients and were associated with 1 death (pulmonary hemorrhage, hypotension).

Summary/Conclusions: The 74% survival rate at Day +70 in patients with VOD/SOS receiving defibrotide within 30 days of starting chemotherapy (81% in patients ≤16 years) is clinically encouraging. Of note is the 66% survival rate in patients with MOD. The defibrotide safety profile was consistent with that previously reported in the overall population of this expanded-access protocol. Support: Jazz Pharmaceuticals.

References:

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2Background: Hepatic VOD/SOS, which may be unpredictable and potentially life-threatening, is typically considered a complication of hematopoietic stem cell transplantation (HSCT), and VOD/SOS with multi-organ dysfunction (MOD) may be associated with >80% mortality. Defibrotide is approved to treat severe hepatic VOD/SOS post-HSCT in the European Union and to treat hepatic VOD/SOS with renal or pulmonary dysfunction post-HSCT in the United States. However, VOD/SOS can occur after chemotherapy without HSCT.

Aims: To perform a post hoc analysis of final data on safety and response to defibrotide in patients developing VOD/SOS after primary chemotherapy without HSCT.

Methods: In an expanded-access protocol for patients with VOD/SOS post-HCT or chemotherapy, with or without MOD (renal and/or pulmonary dysfunction), defibrotide 25 mg/kg/d (4 divided doses of 6.25 mg/kg) was given a recommended ≥21 days after patients provided informed consent. Post-chemotherapy subgroup survival was analyzed post hoc from the day defibrotide was started (days 0–30 after start of chemotherapy) for 70 days (because follow-up data were collected for 100 days post-chemotherapy).

Results: Of 1154 VOD/SOS patients receiving defibrotide, 137 (12%) developed VOD/SOS post-chemotherapy without HSCT. Among the 82 patients (38 with MOD) treated with DF by day 30 after start of chemotherapy, median age was 7.5 years (range, 0–68 years) and 66 (81%) were pediatric patients (≤16 years of age). Among pediatric patients, 15% were age 0–23 months, 74% were 2–11 years and 11% were 12–16 years. Most common primary diseases were acute lymphocytic leukemia (51%), acute myeloid leukemia (13%), and neuroblastoma (6%). Kaplan-Meier estimated survival at Day +70 was 74% overall (95% CI, 63–82%); 86% (49–79%) in patients with MOD and 81% (66–90%) in patients without MOD. By age subgroup, Kaplan-Meier estimated survival at Day +70 was 80% (95% CI, 68–88%) in pediatric patients (Figure 1) and 50% (95% CI, 25–71%) in adults. Adverse events (AEs) were reported in 54/82 patients (66%). Hemorrhagic AEs (≥2%) were pulmonary (6%), epistaxis or mouth (4%), and hematoma (2%). There were 22 (27%) patients with AEs assessed as being at least possibly related to defibrotide, the most common (≥2%) were pulmonary or mouth hemorrhage (4% each) and hematoma (2%). Relat-ed AEs led to discontinuation in 6 patients and were associated with 1 death (pulmonary hemorrhage, hypotension).
Background: GPX4 is a selenoprotein belonging to the family of the glutathione peroxidases, a class of enzymes involved in cellular defence against oxidative stress. This enzyme is essential for life since it is the only peroxidase able to use lipid peroxides as substrate. Mice constitutively lacking GPX4 die at embryonic day 18 due to sepsis and tissue-specific ablation in neurons and T-cells cause neuron degeneration and impaired immune response. Recent studies have identified GPX4 as the main regulator of ferroptosis, an iron-dependent ROS-mediated form of nonapoptotic cell death. Erythrocytes are highly specialized cells that utilize a large amount of iron to bind and deliver oxygen to all tissues. Being constantly exposed to oxygen, erythroid cells need to continuously fight against oxidative stress by expressing a variety of antioxidant enzymes, including GPX4. Iron availability for erythropoiesis depends on systemic iron levels which are regulated via the hepcidin/ferroportin regulatory system. Hepcidin binding to the iron exporter ferroportin reduces systemic iron export regulating body iron levels. In hypoxic conditions the erythroid hormone ErFe suppresses hep- cidin synthesis to provide iron for the elevated erythropoietic demand.

Aims: The aim of this study is to identify how the lack of GPX4 in the hematological compartment affects iron homeostasis.

Methods: Lethally irradiated C57BL/6 female mice were reconstituted with bone marrow cells from Gpx4fl/fl; Rosa26-CreERT2 or Gpx4wt/wt; Rosa26-CreERT2 and allowed to recover for 8 to 10 weeks. GPX4 deletion in the hematopoietic system was induced by feeding tamoxifen citrate for 3 weeks and blood and organs were drawn at 3 and 6 weeks after terminating the tamoxifen-containing diet. Erythroid cells have been analysed in FACS. Serum iron levels have been assessed using the SFBC and UIBC iron kits (Biolabo). Gene expression analysis has been performed using SYBR-green qRT-PCR. Circulating Hepcidin has been measured with a specific murine ELISA kit (Intrinsic Lifesciences). Tissue iron levels have been measured with a colorimetric assay. All animal experiments were approved by and conducted in compliance with institutional guidelines with a specific murine ELISA kit (Intrinsic Lifesciences). Tissue iron levels have been measured with a colorimetric assay. All animal experiments were approved by and conducted in compliance with institutional guidelines.

Results: Compared to Gpx4wt/wt;CreERT2 controls, Gpx4fl/fl;CreERT2 transplanted mice lacking GPX4 in the haematological compartment show a decrease in the number of red blood cells, haemoglobin and haematocrit. Reticulocyte count measurement revealed a strong increase in this population, suggesting that the erythropenia could be due to a block in the reticulocyte maturation. Reticulocyte FACS characterization revealed a shift towards a more immature population while electron microscopy analysis showed an accumulation of unphagocytosed vesicles containing remnants of mitochondria. Analysis of the spleen revealed extramedullary erythropoiesis. The anaemia and the erythropenia trigger a hypoxic signature hallmarkled by an increase in circulating EPO and increased ErFe expression. However, both hepatic mRNA analysis and circulating protein measurement failed to show alteration in hepcidin pro- duction. Analysis of the liver showed an increase in non-heme iron content and in the lipid peroxidation causing an elevated mRNA and protein expression of heme oxygenase 1. Hepatic ferritin and ferroportin are also increased as a consequence of the increased iron content.

Summary/Conclusions: Our data show for the first time that the presence of GPX4 in the haematological compartment is essential for the proper hepcidin downregulation upon ErFe stimulation. This finding opens new insights in the mechanism that regulate hepcidin during hypoxia.
Aims: The main aim of our study is to assess the effects of RAP-011 on different cell types of CDAII patients.

Methods: We measured circulating GDF11 levels in CDAII patients and healthy controls (HC) by western blot (WB). To assess the effectiveness of RAP-011 (provided by Celgene Corporation) in vitro, we established two different cellular models of CDAII: (i) K562 cells stably silenced for SEC23B by Sh-RNA carried in the pSilencer 6.1 vector and (ii) K562 stably overexpressing SEC23B-WT and the two variants, R14W and E109K. In vitro treatment has been performed at 0, 3, and 6 days of erythroid differentiation by hemo+GDF11 in presence or absence of RAP-011 in K562 cells stably silenced for SEC23B.

Results: WB and subsequent densitometric analysis showed an increase of GDF11 in SEC23B silenced K562 cells from 18-fold in SEC23B-WT AUG-mRNA levels in K562 cells starting on day 3 and reaching a maximum on day 6 (p<0.02). Stable silencing of SEC23B in K562 cells led to the establishment of two different clones, Sh-70 and Sh-74, showing amarkereduction of SEC23Bexpression compared to Sh-CTR (85-90% and 60-65%, respectively). At 3 and 6 days of K562 erythroid differentiation by hemo, we observed an increasing expression of pSMAD2 in GDF11-treated cells compared to non-treated ones; interestingly, a reduction of pSMAD2 in RAP-011+GDF11-treated cells was observed.

Summary/Conclusions: We firstly demonstrated the increased levels of GDF11 in CDAII patients. Thus, we used a combined treatment with hemo+RAP-011 in SEC23B silenced K562 stable clones, in order to reproduce the pathologic phenotype of the disease, and to make K562 cells suitable for RAP-011 treatment, as attested by the increased expression of pSMAD2 in GDF11-treated cells. The reduced pSMAD2 in RAP-011+GDF11-treated cells suggests that RAP-011treatment leads to repression of ActRIIA/B pathway, which in turn should increase nuclear levels of GATA1 transcription factor. This action should lead to an increased expression of GATA1-activated genes involved in erythroid development. The evaluation of GATA1 activation is ongoing, as well as the in vitro treatment of K562 stably overexpressing SEC23B-WT, SEC23B-R14W and -E109K.

S812 INTRAVENOUS IRON VERSUS ORAL IRON VERSUS NO IRON WITH OR WITHOUT ERYTHROPOIESISSTIMULATING AGENTS FOR CANCER PATIENTS WITH ANAEMIA: A SYSTEMATIC REVIEW AND NETWORK META-ANALYSIS
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Background: A widely prevalent complication in patients suffering from cancer is the deficiency of haemoglobin-containing red blood cells, referred to as anaemia. While many patients develop anaemia due to an involvement of malignant bone marrow cells, others suffer from so-called chemotherapy/ radiotherapy-induced anaemia. Erythropoiesis-stimulating agents (ESAs) stimulate the production of red blood cells within the bone marrow and have shown to increase Hb levels in anaemic patients. Uncertainties remain regarding the effect of iron supplementation on the fatal consequences of ESA-treatment.

Aims: The aim of this systematic review and network meta-analysis are to evaluate the efficacy of ESAs and iron for the treatment of disease-related as well as therapy induced anaemia in cancer patients.

Methods: Based on an a-priori Cochrane protocol, we developed sensitive search strategies for Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, databases of ongoing trials and conference proceedings (search date 12/2016). We included only randomized controlled trials (RCTs) including anaemic patients of any age with solid and/or haematological malignancy undergoing chemotherapy, radiotherapy or no anti-cancer therapy. We excluded studies including anaemic cancer-patients as a result of surgery or related as well as therapy induced anaemia in cancer patients.

Results: 33 studies including 8 treatments. The given network was not fully connected. Inconsistency could not be tested statistically as no closed loop was included. Thromboembolic events occurred most often in patients treated with ESAs, irrespective of iron supplementation (ESA plus iron vs no treatment/placebo plus no iron: RR 1.79 (95% CI 0.74-4.32) P-score: 0.22, ESA plus no iron vs no treatment/placebo plus no iron: RR 1.90 (95% CI 0.96-3.75) P-score: 0.16). Group comparison analysis showed an increased mean of Hb levels (g/dL) among ESAs irrespective of iron supplementation (group ESA alone=7.1, ESA plus iron=7.5). No relevant heterogeneity was found within the analysed network of four treatments (I²=18.4%). In ESA alone, the treatment could not be tested statistically as no closed loop was included. Further investigation, with regards to iron type and route of administration may yield further distinct results.

Summary/Conclusions: While our analyses show that ESA use increases mortality risk and make thromboembolic events, there is no evidence that iron supplementation alters these risks. However, addition of iron to ESA does further decrease the need for RBC-transfusions compared to ESA alone. Further investigation, with regards to iron type and route of administration may yield further distinct results.

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S813 DIFFERENT IRON SOURCES AND ACQUISITION PATHWAYS SHAPE MACROPHAGES TOWARDS OPPOSING FUNCTIONAL PHENOTYPES
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Background: Iron homeostasis and macrophage biology are closely interconnected. On the one hand, reticulo-endothelial macrophages are central for the regulation of iron homeostasis. The phagocytosis and degradation of senescent red blood cells (RBC) by macrophages enable efficient recycling of iron and the maintenance of systemic iron balance. On the other hand, iron exerts multiple effects on macrophage polarization and functionality. Macrophages exhibit a remarkable functional plasticity, reflected in their capacity to integrate diverse signals from the microenvironment and acquire distinct phenotypes. Macrophage polarization has been shown to dictate the expression of iron-regulated genes and determine cell iron handling.

Aims: Increasing evidence shows that iron availability itself has significant effects on immune effector functions and macrophage polarization. However, it is still unclear how different iron sources and acquisition pathways affect macrophage phenotypes.

Methods: To investigate this aspect, we analyzed both in vivo and in vitro, and compared the phenotypic switching of macrophages induced by different iron sources, including heme and iron, as well as hemolytic or intact RBCs.

Results: Hemolytic RBCs, free heme and iron-dextran treatment in mice shape macrophage polarization towards an M1-like pro-inflammatory phenotype. Splenic and hepatic macrophages from treated mice show iron deposition and increased expression of iron-related genes (ferroportin, ferritin, HO-1). Moreover, in these cells, the expression of M1 markers such as MHCII, CD86 and pro-inflammatory cytokines (TNFa, IL-6, IL-1b) is strongly increased, whereas the expression of M2 markers such as CD206, Arg-1 and IL-10 was significantly suppressed. Consistent results have been obtained treating bone marrow-derived macrophages with hemolytic RBCs, free heme and Fe-NTA. Importantly, the addition of the heme scavenger hemopexin and the iron carrier transferrin or the chelator deferoxamine fully abolish the ability of free heme and iron to trigger M1 polarization. On the contrary, RBC transfusions in mice shape macrophages towards an M2-like anti-inflammatory phenotype. After three transfusions, serum iron and hepcidin levels significantly rise, and tissues as well as macrophages are heavily iron loaded. Macrophages show a drastic suppression of M1 markers and inflammatory cytokines, and induction of M2 markers. Interestingly, repeatedly transfused results in extensive macrophage cell death and new macrophages recruitment in both liver and spleen.

Summary/Conclusions: Collectively, these results suggest that the source and route of iron acquisition have a key role in shaping macrophage phenotype, and demonstrate a dynamic role of iron overload in determining macrophage polarization and function. When iron is provided in the form of free heme or non-transferrin bound iron, it exerts a clear pro-inflammatory effect on macrophages; whereas when provided via a controlled physiological acquisition pathway such as erythropagocytosis, it dampens macrophage immune effect function, being its clearance activity more active.
Our findings have potential implications, on one side, for hemolytic diseases, where RBC hemolysis and elevated circulating heme might promote a detrimental chronic inflammatory state, and, on the other one, for infectious diseases, where free heme and iron, released upon cell damage, might boost inflammation and enhance resistance to infections. Conversely, accelerated RBC clearance, by suppressing macrophage pro-inflammatory response, is rather expected to promote infections in transfused individuals.

**Gene therapy, cellular immunotherapy and vaccination 2**

**S814**

**A PHASE 3 STUDY TO EVALUATE SAFETY AND EFFICACY OF LENTIGLOBIN GENE THERAPY FOR TRANSFUSION-DEPENDENT B-THALASSEMINA IN PATIENTS WITH NON-B0/B0 GENOTYPES: THE NORTHSTAR-2 (HGB-207) TRIAL**

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**Background:** Standard treatment for transfusion-dependent b-thalassemia (TDT) includes regular red blood cell (RBC) transfusions and management of iron overload. Successful allogeneic hematopoietic cell transplantation (HCT) can eliminate RBC transfusions and, eventually, chelation. However, due to transplant-related risks such as graft-versus-host disease (GVHD), as well as donor constraints, HCT is rarely an option for TDT patients. By transferring a functioning copy of the β-globin (HBB) gene into hematopoietic stem cells (CD34+ cells) and re-infusing the modified cells, gene therapy may be an alternative one-time treatment available to all patients with TDT, without risks of GVHD. LentiGlobin gene therapy is an investigational treatment consisting of autologous CD34+ cells transduced with the BB305 lentiviral vector. The Northstar (HGB-204) phase I/II clinical study of LentiGlobin gene therapy for TDT included 18 patients who received LentiGlobin drug product (DP). As of September 2016, all patients in Northstar with non-β0/β0 genotypes and at least 12 months of follow-up stopped transfusions (median total hemoglobin [Hb] 11.2 [range 9.4−12.2] g/dL) and there was >60% reduction in transfusions in patients with a β0/β0 genotype. The safety profile was consistent with autologous HCT. In this initial study, the average number of therapeutic gene copies per CD34+ cell in the DP (i.e. DP vector copy number per diploid genome or DP VCN; median 0.7, range 0.3 to 1.5) correlated with peripheral HbAβ0/β0 (genetically engineered hemoglobin) expression at 6 months (ASH, 2016). In an effort to optimize the proportion of patients able to discontinue blood transfusions to achieve “transfusion independence” in all patients and increase unsupported Hb levels after treatment, the manufacturing process for LentiGlobin DP was modified to increase the DP VCN and the proportion of genetically modified cells. Northstar-2 (HGB-207) is a recently initiated phase 3 study using this new manufacturing process in patients with TDT and a non-β0/β0 genotype.

**Aims:** To evaluate safety and efficacy of autologous HCT with LentiGlobin DP in patients with TDT and a non-β0/β0 genotype.

**Methods:** After providing informed consent, patients 12 to 50 years of age (N=15) will have CD34+ cells collected via mobilization and apheresis. After individualized DP manufacture and satisfaction of release criteria, the patient will receive myeloablative conditioning with single-agent busulfan (starting dose 32 mg/kg/day for 4 days, with target AUC 4300 [range 4000−5000] µM*min) followed by infusion of LentiGlobin DP. Patients will be followed for engraftment, safety and efficacy endpoints for 2 years after infusion; patients will then have the option to enroll in a 13-year follow-up study. The primary endpoint is the proportion of patients who achieve transfusion independence after DP infusion, defined as total Hb ≥9g/dL without RBC transfusions for a continuous period of ≥12 months. Secondary endpoints include time to neutrophil engraftment, adverse events, and biological parameters including VCN in peripheral blood and levels of HbAβ0/β0 over time.

**Results:** As of March 1, 2017, two 20-year-old females with β0/βE genotypes have been treated with LentiGlobin DP in the Northstar-2 trial. The DP VCN was 2.9 and 2.4 copies per diploid genome, respectively. Outcomes in all evaluable patients will be presented.

**Summary/Conclusions:** Results from the Northstar-2 study will provide data on safety and demonstrate the extent to which an increase in LentiGlobin DP VCN yields normalization of total Hb and consistently achieves transfusion independence in patients with TDT of non-β0/β0 genotypes. Optimizing DP VCN has the potential to improve outcomes across all TDT genotypes treated by investigational LentiGlobin gene therapy.

**S815**

**CIS IS A POTENT CHECKPOINT IN NK CELL ANTI-LEUKEMIA IMMUNITY**

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**Background:** The detection of leukemia by natural killer (NK) cells is controlled
from signals from activating and inhibitory ligands and from cytokines such as IL-15.

Aims: We set out to identify the negative regulators of NK cell function in order to understand why immunogenic tumours and leukemia can evade or overcome NK cell detection and killing.

Methods: We used a multidisciplinary approach including RNAseq, Mass Spectrometry, Structual biology, kinase enrichment and activity assays, NK cell in vitro analysis, biochemistry and de novo/experimental tumor/leukemia in vivo models.

Results: We identified cytokine-inducible SH2-containing protein (CIS, encoded by Cish) as a critical negative regulator of IL-15 signaling in NK cells. Cish was rapidly induced in NK cells in response to IL-15, and deletion of Cish rendered NK cells hypersensitive to IL-15, as evidenced by enhanced proliferation, survival, IFN-gamma production and cytotoxicity toward tumors. This was associated with increased JAK-STAT signaling in NK cells in which Cish was deleted. Correspondingly, CISH interacted with the tyrosine kinase JAK1, inhibiting its enzymatic activity and reducing JAK-mediated transcriptional degradation. CISH−/− mice are resistant to leukemia in vivo, and this was independent of MHC-I expression.

Summary/Conclusions: Our data uncover a potent intracellular checkpoint in NK cell-mediated tumor immunity and suggest possibilities for new cancer immunotherapies directed at blocking CIS function.

S816

GENERATION OF MEMORY STEM T CELLS MODIFIED WITH A NOVEL OPTIMIZED CD30-SPECIFIC CHIMERIC ANTIGEN RECEPTOR FOR THE TREATMENT OF CD30+ T-CELL MALIGNANCIES

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Background: Peripheral T-cell lymphomas (PTCL) represent the most aggressive form among non-Hodgkin lymphomas with a very poor prognosis (5-year survival of 30%), demanding innovative novel treatment strategies. Adoptive immunotherapy with chimeric antigen receptor (CAR) engineered T cells has demonstrated its therapeutic potential in advanced hematological malignancies. However, its application to PTCL remains a formidable challenge mainly due to a lack of truly tumor-specific antigens that are not expressed on normal T cells. Anaplastic large T-cell lymphomas (ALCL) and several other subtypes of PTCL express CD30, which is expressed by activated normal T cells but no other healthy tissues. Indeed, brentuximab vedotin, an anti-CD30 antibody-drug conjugate, has shown some clinical efficacy in PTCL and ALCL patients although duration of responses is short in the majority of cases. Here, we developed a refined CD30-CAR T-cell approach to target CD30+ PTCL as a potential novel therapeutic strategy. We selected a novel targeting domain that is unaffected by soluble CD30 protein to prevent blockade of the CD30-CAR in vivo. Moreover, to optimize the therapy by using memory stem T cells (TSCM), we promote engraftment and persistence of CD30-CAR Tc cells after transfer, and we have included an EGFR deletion marker as a safety feature.

Aims: We evaluated the antitumor effect of memory stem T cells (TSCM) genetically modified with a novel CD30-specific CAR that recognizes a membrane-proximal epitope in the CD30 molecule and in CD30+ T-cell lymphoma models.

Methods: A second generation CD30-41BBz-EGFRt CAR was generated using a scFv that recognizes a tumor-cell membrane-proximal epitope of CD30 protein (Nagata S et al. Clin Cancer Res, 2002). Naïve T cells from healthy donors were activated with anti-CD3/CD28 beads in presence of IL-7, IL-15 and IL-2 during 10 days to obtain a TSCM-enriched population (Alvarez G et al. J Transl Med, 2016); on day 2 of culture, cells were transduced with a third-generation lentiviral vector encoding the CD30-CAR. The anaplastic large T-cell lymphoma cell line Karpas 299 was used as tumor model. Cytotoxicity assay was performed at 4 hours at 10:1, 5:1, 1:1 and 1:5 effector/target (E/T) ratios, and the tumor cell death was determined by flow cytometry. Cytokines (IFN-γ and IL-2) were analysed at 24 hours in a 5:1 E/T ratio culture using LumineX technology.

Results: TSCM were the most prevalent T-cell subset at day 10 of culture, representing 84 ± 3.1% of total cells, and the CD30-CAR expression in these cells was 76.9 ± 1.0% in CD4+ TSCM and 77.3 ± 2.0% in CD8+ TSCM. Although CD30 protein was detected in a fraction of activated T cells in culture (CD4+ T cells: 32.4 ± 2.1%; CD8+ T cells: 59 ± 4.3%), lentiviral transduction of TSCM with our CD30-CAR did not compromise their ex vivo expansion (CD4+ CD30-CAR TSCM: 96.0 ± 3.2 fold expansion; CD8+ CAR TSCM: 109.0 ± 4.2 fold expansion). CD8+ CD30-CAR TSCM conferred specific cytotoxic activity and lysis of CD30+ Karpas 299 cells (tumor cell death 1:1 ratio: 92.6 ± 2.4% vs 0% with untransduced TSCM, p<0.001), while control CD30+ target cells (Raji) were not recognized. In addition, CD30-CAR TSCM secreted IFN-γ and IL-2 after stimulation with Karpas 299 cells (IFN-γ: 126.6 ± 18.12 pg/ml vs 5.03 ± 0.16 pg/ml with control targets, p<0.002; IL-2: 20.47 ± 2.3 pg/ml vs 4.06 ± 0.24 pg/ml, p<0.002).

Summary/Conclusions: Collectively, our data demonstrate the potential to generate CD30-CAR T cells with enhanced functional attributes against CD30+ PTCL. TSCM cells can be efficiently transduced and ex vivo expanded with a novel CD30-CAR and confer potent antitumor efficacy against CD30+ PTCL in vivo. Our findings suggest the potential to improve outcome of patients with CD30+ PTCL through adoptive therapy with CD30-CAR modified T cells.

S817

MESENCHYMAL STROMAL CELLS FOR THE TREATMENT OF STEROID-RESISTANT ACUTE GRAFT VERSUS HOST DISEASE: FACTORS INFLUENCING CLINICAL RESPONSES


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Background: The immunosuppressive activity of mesenchymal stromal cells (MSC) have been extensively tested for the treatment of steroid-resistant acute graft versus host disease (aGvHD). However, the factors affecting clinical responses are poorly understood.

Aims: We assessed the impact of MSC treatment on clinical outcomes and investigate factors influencing the response to MSC.

Methods: Data collected from a cohort of 60 patients treated with MSC between May 2008 and December 2014 in the UK were analyzed. Clinical grade MSC were generated from bone marrow aspirates collected from the iliac crest of healthy donors and expanded using platelet lysate. All patients received MSC for the treatment of steroid-resistant aGvHD, defined as failure to respond to high-dose steroids (2mg/Kg methyl-prednisolone) after 6 days. Informed consent was obtained from all patients in accordance with the local ethics committee requirements. Clinical responses to MSC were assessed 1 week after MSC infusion. Patients were defined as: a) Responders if they had a complete response (CR) or partial response (PR), b) Non-Responders if they had stable disease (SD) or progressive disease (PD). We performed exploratory univariate and multivariate analyses to investigate if factors, including baseline characteristics and treatment-related variables, were related to clinical response. The primary outcome was the clinical response to MSC treatment, and secondary outcomes were the time of onset of response, the duration of response, and the toxicity profile.

Results: Patient characteristics are summarized in Table 1.

Table 1.

aGvHD was biopsy proven in 45 patients, while in the remaining patients the diagnosis was clinical and based on the exclusion of other causes. 10, 16 and 1 patients had skin, gut and liver involvement only, respectively. 16 patients exhibited gut and skin, 11 skin, gut and liver, 3 skin and liver and 3 gut and liver. 34 patients received 1 dose, while 19, 6 and 1 were treated with two, three and four doses, respectively. No side effects were observed. 36 patients (60%) responded to MSC. Amongst patients who received multiples doses (26), subsequent doses did not change the status after the first dose (24 responded, 1 did not respond), except from one patient who, although respond-
ing to the first dose, failed to respond to the second one. When we evaluated potential factors for response, organ involvement, age at transplant and the cumulative dose of MSC infused were found statistically significant. Response rate was 67% among patients with involvement of gut, skin or both, but only 22% among those with involvement of the liver (alone or in combination with skin and/or gut). Patients younger than 20 years fared better, with 88% of them responding. Conversely, only 30% and 42% of those aged 20-50 years or older than 50 responded, respectively. Lastly, higher cumulative MSC dose (>3.0x10^6/Kg) was associated with a response in 76%, while none of those receiving less than 1.5x10^6/Kg responded. All 3 factors remained significant in multivariate logistic regression analysis. Patient gender, pre-MSC therapy, interval from transplant or aGvHD diagnosis to MSC treatment and grade of aGvHD did not affect response. The impact of achieving a response 1 week after MSC had a profound impact on the overall survival at 18 months accounting for 59% in responders and 17% in non-responders (log-rank test, p<.001).

Summary/Conclusions: In our cohort of patients, MSC treatment was safe and well tolerated. We conclude that the presence of a response at one week highly impacted on the survival of patients with an otherwise very poor prognosis. Importantly, younger age at the transplant, absence of liver aGvHD involvement and use of higher MSC doses were strong predictors of a response.

S818

CARD9 CONTROLS DECTIN-1-INDUCED T-CELL CYTOTOXICITY AND TUMOR GROWTH IN MICE

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Background: Activation of the C-type lectin receptor Dectin-1 by beta-glucans triggers multiple signals within dendritic cells (DCs) that result in activation of innate immunity. While these mechanisms can potently prime CD8+ cytotoxic T cell (CTL) responses without additional adjuvants, the Dectin-1 effector pathways that control CTL induction remain unclear.

Aims: Aims of this study were: To define details of the intracellular signalling pathway responsible for cross-priming of a CTL response after activation of the C-type lectin receptor Dectin-1. To analyze whether identified signalling molecules were indispensable for antitumor immunity. To analyze whether NK cells played a role in antitumor immunity after Dectin-1-mediatated CTL induction.

Methods: We used in vitro coculture between DCs (wildtype vs gene deficient) and CD8 T cells to define signalling components of Dectin-1 induced CTL cross-priming. We used WT and gene-deficient mice to define the signalling pathway of Dectin-1 induced CTL crosspriming in vivo and to test the role of this pathway for antitumor immunity by challenging mice with B16-Ova tumor cells intravenously, with or without depletion of CD8 T cells or NK cells, respectively.

Results: Here we demonstrate that Dectin-1-induced CTL cross-priming in mice does not require inflammasome activation but strictly depends on the adapter protein Card9 in vitro. In vivo, Dectin-1-mediated Card9 activation after vaccination drives both expansion and activation of antigen-specific CTLs, resulting in long-lasting CTL responses which are sufficient to protect mice from tumor challenge. This Dectin-1-induced antitumor immune response was independent of natural killer (NK) cell function and completely abrogated in Card9-deficient mice. Thus, our results demonstrate that Dectin-1-triggered Card9 signaling but not inflammasome activation can potently cross-prime antigen specific CTLs, suggesting that this pathway would be a candidate for immunotherapy and vaccine development (Figure 1).

Figure 1.

Summary/Conclusions: We identify Card9 as central regulator of Dectin-1-induced cross-priming of cytotoxic T cells (CTLs) in mice. These antigen specific CTLs mediate potent antitumor immunity independent of inflammasome activity and NK cells. This pathway is a candidate for immunotherapy and vaccine development.
Acute lymphoblastic leukemia - Biology

E819
PRECLINICAL COMBINATION OF A NOVEL IRE1 RNASE INHIBITOR MKC-866 AND TYROSINE KINASE INHIBITION ACTS SYNERGISTIC IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: The role of the Unfolded Protein Response (UPR) in BCR-ABL+ ALL has been extensively studied, proving the importance of Notch signalling as well as its contribution to chemotherapy resistance. The IRE1-XBP1 branch to be required for leukemic cell survival. However, a therapeutic strategy involving UPR inhibition that possesses translational impact is yet to be identified.

Aims: In this study we aim to identify a potential synergistic effect of simultaneous pharmacological inhibition of IRE1 and BCR-ABL1 in 1B-ALL patient samples. Methods: To study the link between IRE1-XBP1 axis of UPR and BCR-ABL1, we utilized both pharmacological and genetic approaches. 1) We tested the effect on proliferation and viability of pharmacological IRE1 inhibition (using MKC-866) alone and in combination with Tyrosine Kinase Inhibitors (TKI, using Imatinib or Nilotinib) on BCR-AVL1 human ALL cell lines, SUP-B15 and TOM-1. The cell lines were also co-cultured with immortalized tetrMSCs to test the chemo-protective effect of bone marrow stromal cells (BMsCs) on leukemia cells. 2) We tested whether genetic knock-down of XBP1 could sensitize cells towards the effect of Imatinib and Nilotinib. To this end, primary murine pre-B cells from conditional XBP1fl/+ mice were transduced with BCR-ABL1 construct and with either inducible cre or empty vector.

Results: IRE1 inhibitor MKC-866 (MKC) in combination with either Imatinib (IM) or Nilotinib (NL) showed enhanced capacity to arrest proliferation and to induce cell death in BCR-ABL1 ALL cell lines compared to single treatments, after 3 days incubation (Viable SUP-B15: MKC 30µM 94.9%±0.1, IM 10µM 78.7±0.4, Combination 78.7±0.4; MKC 30µM 94.1±0.07, IM 10µM 89.9±0.2, Combination 94.1±0.07). With BCR-ABL1 ALL cell lines, we confirmed a striking synergistic effect. Successively, to exclude any possible off-target effect at the basis of the observed synergism, we used a genetic approach to block IRE1-XBP1 signaling in vitro. B-cell precursors from Xbp1fl/+ mice, instead of Xbp1fl/fl, were used in order to warrant a basal signal of XBP1, as present during pharmacological inhibition. After transfections with BCR-ABL1, and either cre or the empty vector, we could observe that heterozygous deletion of XBP1, induced by 4OH, significantly increased TKI-induced cell death, after 3 days incubation (4OH 1µM: 47.5%±13.0, IM 1µM: 70.8±1.7, IM+4OH: 18.3±2.7, NL 0.5µM: 65.2±0.3, 4OH+NL: 8.6±1.2). Finally, we showed whether the tested drugs combinations were still effective in presence of BMSCs. It’s known that BMSCs are a critical component to escape TKI-induced cell death in pre-B leukemia and that IRE1-XBP1 is responsible for chemoresistance in many different cancer types, although this role has never been confirmed in BCR-ABL1+ cells. To shed light on this aspect we co-cultured either SUP-B15 or TOM-1 cells with tetrMSCs, and while the stroma was capable to block Nilotinib-induced cell death, after 5 days incubation (in SUP-B15, NL 5µM in standard culture 28.7±0.4), this protective activity was partially abrogated upon treatment with IRE1 inhibitor. On the other hand, MSCs were not able to reverse IM effect on cell viability.

Summary/Conclusions: Overall, our data demonstrate that simultaneous inhibition of BCR-ABL1 and IRE1 branch of UPR exerts a potent effect in vitro, by acting synergistically on BCR-ABL+ ALL cells. This provides basis for a clinical application of a combined targeted therapy.

E820
HIGH-THROUGHPUT COPY NUMBER PROFILING IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA USING MULTIPLE LIGATION-DEPENDENT PROBE AMPLIFICATION IN COMBINATION WITH NEXT-GENERATION SEQUENCING

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Background: Development, progression and resistance of pediatric acute lymphoblastic leukemia (pALL) are widely associated with recurrent copy number abnormalities (CNAs). Multiplex ligation-dependent probe amplification (MLPA) is an established technique to screen CNAs, thus providing valuable information for risk assessment in pALL; however, the number of simultaneously analyzable genomic loci is limited to 55-60.

Aims: To introduce and test a high-throughput, high-resolution and comprehensive disease-relevant CNA profiling approach applicable to all subtypes of pALL.

Methods: A new digitalMLPA (dMLPA) technique has been developed which combines the advantages of MLPA and next-generation sequencing (NGS), massively improving the number of genomic targets that can be analyzed for their copy number in a single reaction. Bone marrow samples from 58 patients with pALL were analyzed using this novel assay targeting ~470 genomic loci. To introduce the method and to test the thoroughness of the data, ~200 digital karyotyping probes covering each chromosome arm.

Results: CNAs directly indicating structural or whole chromosome aberrations or indirectly referring to gene fusions were detected in 93% of patients, in 44/48 pre-B ALL and 10/10 pre-T ALL cases. Among patients with CNAs, recurrent aberrations specifically affecting putative driver genes varied between 0 and 11 CNAs per patient (median: 175). E819 Markers detected in pre-B and pre-T ALL, respectively, followed by CSDK2A/B, PAX5, RB1, VPREB1, MLT3, CD200/BTLA, TLR1XR1, IKZF1, CASP8AP2, Pten, RUNX1, BTG1, TP53, IKZF3, EZH2, NF1, NRG3, RAG2 and the PAR region genes in pre-B ALL cases. E820 Further, in pre-T ALL cases, TP53, MLST, FMP2W, PHIP6, LEP1, CASP8AP2, MYB, RB1, TP53 in pre-T ALL were observed. In pre-B ALL fusions were also observed in T-ALL cases while in one in BCR-ABL1 pre-B ALL patient, the copy number profile correctly indicated the presence of an extra Ph-chromosome. dMLPA resulted in a 99.3% of those with MLPA mixes containing probes with different ligation sites for a subset of the genes. The increased resolution of dMLPA (i) allowed the detection of subclonal aberrations with an improved efficacy and confidence as compared to conventional MLPA and (ii) enabled a more patient-specific characterization of CNAs, e.g. by revealing 15 different deletion patterns across 23 samples harboring del(9p). In addition to genomic lesions specifically influencing putative or proven driver relevant genes, 24 structural and 134 whole chromosome aberrations were detected genome-wide which was strongly facilitated by the inclusion of ~200 digital karyotyping probes covering each chromosome arm.

Summary/Conclusions: A novel NGS-based method has successfully been introduced for high-resolution profiling of CNAs in pALL. dMLPA is a robust, fast and cost-effective technique; its input DNA requirement (20ng) is similar to those of other low-input NGS protocols and lower than the requirement for MLPA. Due to its targeted approach, data analysis is computationally less demanding as compared to most NGS methods. The number of genomic sites analyzed is many orders of magnitude higher than the diversity achievable with conventional MLPA. Due to its specific probe composition, dMLPA allows both high-resolution analysis of genomic driver regions and a genome-wide detection of aneuploidies and large CNAs.

E821
CRITICAL ROLE FOR NOTCH SIGNALLING IN B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA (B-ALL) DRUG RESPONSE

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Background: B-cell precursor acute lymphoblastic leukemia (B-ALL) is the leading cause of cancer-related death in children and young adults. There is still a need of more efficient therapies for the subset of refractory patients. Our group has previously shown that Notch-3 and Notch-4 promote human B-ALL cell survival in presence of stromal cell support. However, the prognosis value of Notch signalling as well as its contribution to chemotherapy resistance have not yet been investigated.

Aims: In this study we used B-ALL cell lines and samples from new diagnosed B-ALL patients to analyse the contribution of Notch signalling to B-ALL pathogenesis in terms of prognosis, proliferation survival and drug response in vitro and in mice xenograft models of B-ALL.

Methods: B-ALL cell lines were obtained from ATCC, while B-ALL primary cells were obtained from bone marrow or peripheral blood of 30 B-ALL patients. Flow cytometry and western immunoblotting were used to study the expression of Notch receptors and ligands. Drugs used were Cytarabine (Ara-C), Dexamethasone (Dexa) and Doxorubicin (Doxo) alone or in combination with Notch inhibitors (GSIs), and Notch transcription factor inhibitor (SAHM1). Mice xenograft model of B-ALL were obtained by injecting the B-ALL line RS4;11 in
NOD/Shi-scid/I-L2rnull mice (NOG). Cell viability was evaluated by Annexin-V/PI and MTT assay; proliferation was assessed through CFSE dilution.

Results: Western blot and flow cytometric analysis showed that B-ALL cell lines as well as primary blast cells displayed the same Notch expression pattern consistent in low expression levels of Notch2 and Jagged1, high expression levels of Notch1, Notch3, Notch4, Jagged2, DLL3 and DLL4. Notably, in primary blast cells deriving from patients, the expression of Notch3, Notch4, Jagged2, DLL3 and DLL4 was significantly higher in the cases refractory to treatment as compared to patients achieving complete remission, thus suggesting that Notch signalling could be involved in the response to chemotherapy. In line with this hypothesis, we found that the treatment in vitro of B-ALL cell lines with Ara-C or Dexa down regulates the expression of Notch receptors. This down regulation was also observed in human CD19+ blast cells isolated from bone marrow of recipient mice treated with Ara-C compared to cells isolated from not treated mice. In addition, Notch inhibitors significantly improved in vitro the cytotoxicity of Ara-C and Dexa towards B-ALL. Finally, we verified the administration to mice of a pan Notch inhibitor, i.e. the GSI XII, significantly lowered the CD19+ leukemic burden in the bone marrow of recipient mice, potentiating anti-leukemic effect of Ara-C.

Summary/Conclusions: In this study we used both in vitro and in vivo assays to highlight the prognostic value of Notch expression in B-ALL, as well as its critical role in B-ALL cell survival and response to chemotherapy. We also demonstrated that Notch inhibitors were able to improve Ara-C-mediated reduction of blast cells in bone marrow, revealing that Notch signalling is a possible therapeutic strategy to eradicate minimal residual disease in B-ALL.

E822

REGULATION OF NOTCH AND WNT SIGNALING PATHWAYS BY NRARP IN T-CELL ACUTE LYMPHOBlastic LEUKEMIA

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Background: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematological malignancy. Although the outcome of T-ALL patients has improved over recent years, the poor prognosis of patients with resistant or relapsed disease is still a major concern. Even though NOTCH is a known driver in T-ALL, its inhibition cannot be efficiently achieved with the drugs currently available, due to their weak therapeutic effects and severe toxicity. We have shown that loss of NRARP expression can contribute to the pathogenesis of T-ALL.

Aims: To investigate the role of NRARP in human T-ALL cell growth and survival and its therapeutic potential in T-ALL.

Methods: mRNA and protein expression were determined by real time-PCR and western blot analyses. in vitro functional evaluation of NRARP in T-ALL cell lines was performed by flow cytometry analysis of proliferation and viability upon NRARP overexpression using lentiviruses.

Results: We started by characterizing NRARP expression in human T-ALL cell lines and compared it with the expression of NRARP in human thymocytes. We found that NRARP protein levels are significantly increased in T-ALL cells. This result, although consistent with the fact that NRARP is a transcriptional target of NOTCH, suggests that NRARP is not sufficient to block NOTCH oncogene signals. To test this hypothesis, we overexpressed NRARP in human T-ALL cell lines. Curiously, NRARP overexpression blocks the expansion of the T-ALL cell lines that display NOTCH1-activating mutations but promotes the expansion of the T-ALL cells without NOTCH1 mutations. Although in both cell types (WT and NOTCH1-mutated) NRARP overexpression blocks NOTCH signaling, in NOTCH1-WT T-ALL cells we observe an increase in c-Myc expression. Consistent with these results, NOTCH1-WT NRARP overexpressing cells are more sensitive to 4Q1, a small-molecule bromodomain inhibitor that targets c-Myc. NRARP is known to positively regulate LEF1, a DNA binding transcription factor acting downstream of WNT. Thus we sought to investigate the impact of this pathway on NRARP expression. Very interestingly, our results show that in NOTCH1-mutant cell lines NRARP overexpression results in the down-regulation of the WNT signalling pathway while in NOTCH1-WT T-ALL cells results in its up-regulation.

Summary/Conclusions: Taken together our results suggest that NRARP may play a dual role in T-ALL pathogenesis, regulating both NOTCH and WNT pathways, with opposite functional effects on leukemia cells depending on NOTCH mutational status and signaling levels. This dual role may have important biological and therapeutic implications.

E823

ETV6/RUNX1-LIKE ACUTE LYMPHOBlastic LEUKEMIA: A NOVEL B-CELL PRECURSOR LEUKEMIA SUBTYPE IDENTIFIED BY THE CD27/CD44 IMMUNOPHENOTYPE

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Background: We have shown previously that ETV6/RUNX1-positive acute lymphoblastic leukemia (ALL) is distinguishable from other ALL subtypes by CD27pos/CD44low-neg immunophenotype. During diagnostic immunophenotyping of 573 childhood B-cell precursor ALL (B-ALL), we identified eight cases with this immunophenotype among ‘B-other ALL’ (B-ALL cases negative for hyperdiploidy, ETV6/RUNX1, TCF3/PBX1 and BCR/ABL1 fusion genes and KMT2A rearrangements).

Aims: We aimed to characterize their genetic and biological background, to reveal to what extent they resemble ETV6/RUNX1-positive ALL and to elucidate whether they may present new therapeutic strategies.

Methods: We utilized microarrays to study the gene expression profile (GEP) and biological similarity of the B-ALL subtypes. Five ETV6/RUNX1-positive and five hyperdiploid ALL cases were analyzed using microarrays in parallel to seven CD27pos/CD44neg cases as a control. Microarray data from all 17 B-ALL cases were combined with data from an independent Italian cohort of 291 BCP-ALL cases (including ETV6/RUNX1-positive, BCR/ABL1-positive, TCF3/PBX1-positive, KMT2A-rearranged, hyperdiploid and B-other ALL cases) whose specimens were analyzed using the same microarray. To study the genomic background, we performed comprehensive profiling using single nucleotide polymorphism (SNP) arrays and whole exome and whole transcriptome sequencing (WES and RNAseq).

Results: In the hierarchical clustering based on GEP all five ETV6/RUNX1-positive cases and 5 of 7 CD27pos/CD44neg B-other cases clustered within the ETV6/RUNX1-positive cluster. These B-other cases thus exhibited a combination of characteristics of ETV6/RUNX1-positive ALL, namely polygenetic features and hyperdiploidy. Several recurrent genetic alterations were found in the ETV6/RUNX1-positive ALL cases, but no particular genetic changes were identified in the CD27pos/CD44neg B-other cases. In the ETV6/RUNX1-positive ALL cases, the sole chromosome 23 rearrangement detected was translocation t(2;5)(p11;p15) (in two cases) and novel t(1;4)(p13;q31) (in one case). The latter rearrangement was also observed in one CD27pos/CD44neg B-other case.

Conclusion: ETV6/RUNX1-positive ALL is a distinct disease entity with a unique genetic and biological background. The clinical utility of the CD27pos/CD44neg immunophenotype for the identification of ETV6/RUNX1-positive ALL cases is confirmed by its high specificity and sensitivity.
and KIZ1. In conjunction with the single published study, our study establishes the ET6 lesion as the only common genetic aberration and thus the most likely key driver of ET6/RUNX1-like ALL.


E824
Abstract withdrawn.

E825
GENETIC ALTERATIONS IN CHILDREN WITH T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA IN TAIWAN
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Background: The leukemogenesis of T-cell acute lymphoblastic leukemia (T-ALL) involves multistep processes of genetic alterations.

Aims: We aimed to determine the genetic alterations including common fusion transcripts, overexpression of T-cell transcription factor oncogenes and deletion or mutations of targeted genes in pediatric T-ALL in Taiwan and their impact on outcomes in those treated with TPOG-ALL2002 protocol.

Methods: Between 1995 and 2015, bone marrow samples from 102 children (<18 years old) consecutively diagnosed with T-ALL were examined. SIL-TAL, MLL-ENL, and CALM-AF10 transcripts were detected by RT-PCR assays. RT-PCR with TaqMan assays were used to measure the expression of HOX11, TAL1, and LYL1 oncogenes expressed as normalized copy number (NCN) to ABL internal control gene. TAL1 overexpression was defined as NCN > the lowest level of SIL-TAL positive samples. Overexpression of HOX11 and TAL1 was defined as NCN > the upper limits of the 50 normal bone marrow controls. Mutations of NOTCH1, FBXW7, PHF6, JAK1, JAK2, RUNX1, WT1, NRAS, and KRAS genes were analyzed by PCR-based assays followed by direct sequencing. P16 deletion was determined by PCR or multiplex ligation probe amplification (MLPA), PTEN and PHF6 deletions, MYB duplication and NUP214-ABL1 overexpression were defined as NCN > the upper limits of the 50 normal bone marrow controls.

Results: The frequency of SIL-TAL fusion transcript was 16.2%, MLL-rearranged 5.1%, CALM-AF10 1.0%, and no NUP214-ABL1. The frequency of NOTCH1 mutations was 46.9%, FBXW7 13.0%, RUNX1 5.2%, WT1 6.3%, NRAS 6.2%, KRAS 2.1%, and no JAK1 or JAK2 mutations. P16 deletion was present in 56.2%, PTEN in 11.1%, PHF6 deletion/mutation in 13.4%, and MYB duplication in 4.8%. Overexpression of TAL1 was present in 46.5%, 22% for LYL1, and 9% for HOX11. The correlation among the genetic alterations showed that LYL1 overexpression occurred more frequently in P16 wild-type compared with P16-deleted patients (P=0.003) and absence of SIL-TAL transcript was significantly associated with absence of LYL1 overexpression (P=0.018). A comparison of outcomes was made according to the state of each genetic abnormality. NOTCH1 mutations conferred a favorable overall survival (OS) (P=0.025), PHF6 deletion/mutation conferred an inferior OS (P=0.030). PTEN deletion was associated with shorter relapse-free survival (RFS) (<0.0001) and OS (P<0.0001). Status of other gene mutations, deletion or duplication did not influence the RFS or OS. TAL1 overexpression predicted a higher risk of relapse (37% vs 21%, P=0.006), an inferior RFS (P=0.002) and OS (P=0.025) whereas HOX11 or LYL1 overexpression had no prognostic impact. Multivariate analysis, including mutation status, reached statistical significance for an independent predictor of OS (HR 0.167, P=0.012), PHF6 deletion/mutation was an independent unfavorable predictor for OS (HR 4.596, P=0.006), and PTEN deletion was also an independent predictor for both RFS (HR 29.493, P=0.007) and OS (HR 15.830, P=0.003). TAL1 overexpression was an independent risk factor for both RFS (HR 3.989, P=0.014) and OS (HR 2.701, P=0.047).

Summary/Conclusions: The present study showed that LYL1 overexpression was negatively associated with SIL-TAL or P16 deletion. PHF6 deletion/mutation, PTEN deletion, and TAL1 overexpression were the independent predictors of adverse outcomes. (Grants support: CORPG3C0201, MMH-E-105-09, NSC-101-2314-B-195-004-MY2, and Tyre Foundation)

E826
COMPUTATIONAL METHODS TO FIND NEW THERAPEUTIC TARGETS IN ALL, SYSTEMATICAL IDENTIFICATION OF ESSENTIAL GENES
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Background: Deletion of chromosomal material is a hallmark of cancer genomes. While these lesions primarily target tumour suppressor genes, neighbouring genes are frequently deleted in parallel. Loss of one chromosome (haploinsufficiency) of a neighbouring gene that is essential for the survival of the cancer cells may constitute potential therapeutic targets in that the cancer cells may be selectively sensitive to further suppression of the function of that gene. Identifying such vulnerabilities is one of the current challenges in cancer genomics. We show that vulnerabilities in cancer cells can be identified by comparing pattern recognition techniques to a copy-number dataset. This approach will identify genomic regions with potential essential genes. In these regions these can be evaluated downstream by genome editing techniques to find novel targets for treatments. Using pattern recognition techniques to find essential genes is a straight-forward, easily applied and non-time-consuming method compared to genome wide experimental approaches.

Aims: Develop a computational framework to find regions with potential essential genes from copy-number data, with a primary focus on hematological malignancies and in particular ALL.

Methods: Our computational framework first selected regions of the tumour genome with heterozygous, but not homozygous, deletion. In sections flanking these regions we scanned for linear increases in homozygous deletion frequency. Genes near the start of these increases that have more than one case with homozygous deletion are discarded. Remaining genes were scored by calculating a line of best fit using the least square method towards the nearby peak in homozygous deletion. We sorted the results by settings cut-offs for the slope, amplitude and correlation coefficient of the linear regression line. Genes with the highest scores were then manually evaluated by comparing to known mean copy-number loss dependence score from other data-sets, by graphical inspection, and by investigating the function of their known orthologues. Our framework analysed contains copy-numbers from tumour samples matched to normal blood samples or normal tissue from the same donor. To validate the essentiality of genes in the discovered regions we used pooled CRISPR/Cas9 editing in ALL cells with and without a deletion of the driving tumour suppressor.

Results: Our framework identified several regions with potentially essential genes around well-known tumour suppressors. The strongest signals in the data set were located around the tumour suppressor CDKN2A. Downstream analysis with pooled CRISPR/Cas9 editing in ALL cells with and without a CDKN2A deletion provided evidence for the essentiality of several genes in the identified region, including one gene that was essential only in CDKN2A-deleted cells.

Summary/Conclusions: In conclusion, we explored a computational approach to identify regions with essential genes in copy-number datasets. Application of our approach to real data showed several regions with essential gene candidates around well-known tumour suppressors, indicating the framework works. Downstream genome-editing experiments in model cell-lines provided further evidence for the essentiality of some genes found in such identified regions. While we cannot yet draw conclusions on whether some of these genes are viable therapeutic targets it allows for informed guesses on limited sets of genes for further focused analysis in hematological model cell-lines.
CRISPR v2 plasmid to produce lentiviral vectors encoding PRDX1-specific sgRNA and Cas-9 and used them to generate BV-173 cells with PRDX1 genomic deletion. Proliferation rate was evaluated by trypan blue exclusion method. Cytostatic/cytotoxic effects of TXN-family enzymes inhibitors, such as adenanthin (ADE), auranofin (AUR) and SKO53 were assessed by MTT viability assay and by detection of propidium iodide-positive cells in flow cytometry.

**Figure 1.**

Results: We have found that B-ALL cell lines exhibit significantly higher levels of ROS as compared to normal B cells isolated from human tonsils (Fig.1A). In accordance with this observation, our analysis of TXN antioxidant enzymes gene expression in B-ALL cell lines showed their upregulation (Fig.1B). Analysis of deposited data revealed that PRDX1 expression level is the highest in B-ALL among the other types of leukemia (Fig.1C). Moreover, we have observed elevated expression of PRDX1 in malignant lymphoblasts derived from pediatric patients at both RNA and protein levels. Genomic deletion of PRDX1 in BV-173 cells leads to suppression of their proliferation rate, comparing to parental cells and cells transduced with mammalian non-targeting sgRNA. These results allow us to suspect that PRDX1 may play growth-supporting role in these cells. Targeting TXN-family enzymes was also performed with the use of various small molecule inhibitors. Both B-ALL cell lines and primary cells are sensitive to PRDX and TXN inhibitors, which reduce cell viability in dose-dependent manner.

**Summary/Conclusions:** All the above results suggest that targeting TXN antioxidant system may exert desirable anticancer effects in the treatment of B-ALL. Inhibitors of TXN-family enzymes can be considered as putative agents to use in combination with classical drugs and improve existing therapeutic approaches. Further studies are underway.

**E828**

RNA-BINDING PROTEIN IGF2BP1 PROMOTES SURVIVAL OF ET6V/ RUNX1 LEUKEMIA CELLS

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**Background:** The IGF2 mRNA binding protein 1 (IGF2BP1, other aliases IMP-1 (IMP1), CRD-BP (CRDBBP), ZBP-1 (ZBP1), and VICKZ1) belongs to a family of regulatory RNA-binding proteins with an oncotelic expression pattern. IGF2BP1 has also been identified to be exclusively specific for ET6V/RUNX1-positive acute lymphoblastic leukemia (ALL) but biological significance of IGF2BP1 overexpression has not been thoroughly investigated to date (Andersson, Olofsen et al. 2005; Stoškus, Gineikiene et al. 2011). We have recently contributed by reporting that ET6V/RUNX1 transcript is a target of RNA-binding protein IGF2BP1 in t(12;21)(p13;q22)-positive ALL, suggesting a role of IGF2BP1 in ET6V/RUNX1-mediated leukemogenic events (Stoškus, Vaitkevičienė et al. 2016).

**Aims:** To define the biological significance of IGF2BP1 overexpression in t(12;21)(p13;q22) ET6V/RUNX1-positive ALL.

**Methods:** In this study we have used stable sublines with downregulated IGF2BP1 from our previously published study (Stoškus, Vaitkevičienė et al. 2016). Dynamics of viable cell population was assessed by flow cytometry using 7-AAD staining (BD Biosciences) following 72 hrs culture in complete medium. An EdU flow assay (Thermo Fisher Scientific, TFS) was used to assay DNA replication in proliferating cells. Spontaneous and doxorubicin (Doxo), staurosporine (STS), and STAT3 selective inhibitor S3i-201 (all from Santa Cruz Biotechnology) induced cell death rates were determined by Annexin V (TFS) and 7-AAD staining. All samples were analyzed on Accuri C6 cytometer (Accuri Cytometers) using CFlow Plus and FCS Express software (De Novo Software). IGF2BP1, ET6V/RUNX1, and STAT3 RT-qPCR was performed essentially as reported previously (Stoškus, Gineikiene et al. 2011). Statistical analyses performed using GraphPad Prism software (GraphPad Software).

**Results:** Downregulation of IGF2BP1 by 2-fold have rendered into approximately 2-fold lower population growth rate, increasing levels of spontaneous cell death in dynamics, and modest yet statistically significant attenuation of cell cycle progression (35.13% vs 40.40%, p<0.0001). Data from treatment with 50 nM of Doxo, 250 nM of STS suggest that IGF2BP1 downregulation has no effect on pharmacological effectiveness of these drugs. In contrast, IGF2BP1-downregulated cells are more sensitive to pharmacological inhibition of STAT3 even upon treatment with suboptimal 25 µM concentration of S3i-201. Lastly, we have probed if STAT3 transcript levels could be sustained by IGF2BP1 protein as in agreement with previously reported (Stohr, Kohn et al. 2012) and our unpublished insights from anti-IGF2BP1 RNA immunoprecipitation datasets. Correlation analysis of RT-qPCR data have confirmed these assumptions as downregulation of IGF2BP1 expression have resulted in a decrease of ET6V/RUNX1 mRNA (r2=0.8253, p<0.001, slope 0.9459) and also STAT3 transcript levels (r2=0.7709, p=0.002, slope 0.6346). These data suggest that STAT3 transcript is also a potentially regulated by RNA-binding protein IGF2BP1 in t(12;21)(p13;q22)-positive ALL model cells (Fig 1).

**Figure 1.**

**Summary/Conclusions:** We provide evidence that IGF2BP1 promotes survival of t(12;21)(p13;q22)-positive ALL model cells through cell cycle progression and preventing spontaneous cell death. Potentiation of ET6V/RUNX1®STAT3 signaling axis is one of the possible mechanisms responsible for this phenotype as IGF2BP1 maintains appropriate levels of primarily ET6V/RUNX1 and also STAT3 mRNAs. Further studies are clearly warranted to further delineate the role of IGF2BP1 in t(12;21)(p13;q22)-positive ALL (Stoškus, Eidukaitė et al. 2016).

**E829**

6-MERCAPTOPURINE PROMOTES ENERGETIC FAILURE IN LEUKEMIC T-CELL LINE JURKAT

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**Background:** 6-Mercaptopurine (6-MP) is a thiopurine drug with antiproliferative effects by blocking purine synthesis. 6-MP is largely prescribed for the treatment of childhood acute lymphoblastic leukemia (ALL). Recent evidence...
suggest that 6-MP inhibits the phosphatidyl-inositol 3 kinase (PI3K)/ mammalian target of Rapamycin (mTOR) signaling pathway and modulates the transcriptional activity of hypoxia inducible factor 1α (HIF-1α). As mTOR and HIF-1α are key mediators of metabolic reprogramming in cancer and normal T cells we hypothesized that 6-MP can impact cellular metabolic remodeling through its action on nucleotide synthesis. Metabolic reprogramming fosters glycolysis, glutaminolysis and nucleotide synthesis to sustain growth and proliferation, a key feature of cancer cells. This metabolic switch is regulated by metabolic checkpoints, including mTOR, AMP-activated protein kinase (AMPK) and the oncogenes Myc and HIF-1α.

Aims: Our objective is to study the impact of the antiproliferative molecule 6-mercaptopurine (6-MP) harboring Treg cells on glucose uptake or on glucose transporters (Glut1 or Glut3, SLC2A1 or SLC2A3) and nucleotide synthesis after 24, 48 and 72 hours of treatment. In addition, 6-MP inhibits the expression of the metabolic checkpoints mTOR, HIF-1α and Myc after 24, 48 and 72 hours of treatment. 6-MP also decreases glucose and glutamine oxidation after 48 hours of treatment by 60% and 35%, respectively, suggesting that 6-MP inhibited TCA (tricarboxylic acid cycle) and OXPHOS (oxidative phosphorylation). The production of lactate, a marker of aerobic glycolysis, is significantly decreased by 30% after 6-MP treatment for 48 hours, meaning that aerobic glycolysis is also inhibited. However, 6-MP has no effect on glucose uptake or on glucose transporters (Glut1 or Glut3, SLC2A1 or SLC2A3) and Myc main expression, suggesting that 6-MP metabolic effects are not linked to glucose uptake.

Summary/Conclusions: In conclusion, our findings offer new insights on the cellular effects of 6-MP treatment by promoting an early energetic stress that influences proliferation and raise apoptosis in leukemia T cells. Interestingly, the inhibition of the metabolic checkpoints (mTOR, HIF-1α, Myc) and the diminution of glycolytic and glutaminolytic fluxes by 6-MP treatment provide an original approach to better understand the cellular effects of 6-MP treatment.

E831
PROFILING OF RECURRENT COPY NUMBER ALTERATIONS IN RELAPSED ADULT B CELL PRONOUNC CELLCURSOR ACUTE LYMPHOBLASTIC LEUKEMIA
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Background: The survival rate of relapsed adult acute lymphoblastic leukemia (ALL) is around 10%. Aims: We looked for recurrent Copy Number Alterations (CNA) in relapsed adult B cell progenitor ALL (B-CP-ALL) to shed light into the molecular mechanisms of relapse.

Methods: BM or PB samples with at least 30% of blasts from 31 adult BCP-ALL patients at 1st relapse and, of them, 21 paired diagnosis and relapse samples were analysed by MLPA (MRC-Holland, The Netherlands). 19 out of these 21 paired samples were analysed by SNP array with CytoScan HD chips (Affymetrix, Santa Clara, California, USA). True CNA were considered when encompassed a minimum of 25 markers, and 25 markers and 220Mb for CN-LOH.

Table 1.
Results: With a median follow up of 12.43 [2.4-30.3] months, the median OS of the 31 patients at first relapse was 7.9 months, [2.4,13.8]. The OS of patients at first relapse was significantly lower in those having more than 3 CNA by MLPA (median ≤3 CNA 9.7 months [0-20.7] vs median >3 CNA 4.2 months [0.6-7.8], p=0.042). CDKN2A/B deletion was the most common CNA observed at relapse (16/31, 52%) and most of these deletions were homozygous (12/16, 75%). One patient had CDKN2A/B deletions homozygous more frequent at relapse (from 8 heterozygous CDKN2A/B deleted patients at diagnosis, 7 became homozygous at relapse, p=0.070). SNP arrays detected 554 CNA (409 DEL, 125 DUP and 20 LOH) in 34 samples of 19 patients. At diagnosis (n=16 patients) the mean number of CNA was 12.5 (9.8 DEL, 2.3 DUP and 0.4 LOH), while at first relapse (n=13 patients) was 17.8 CNA (12.6 DEL, 4.2 DUP and 1 LOH) and in second relapse (n=5 patients) was 21 CNA (14.6 DEL, 6.4 DUP and 0 LOH)(p=0.007). All matched diagnosis and first relapse samples (available for 10 patients) showed common CNA. In 6/10 cases some of CNA were retained from diagnosis while others were acquired or lost at relapse (suggesting a dynamic process of deletion maternal clones). In addition, some CNA were acquired and new CNA at relapse (indicating an evolution from diagnosis clone) and 1/10 showed the same CNA signature at relapse (suggesting a primary resistance of the diagnosis clone). Gene ontology analysis showed a significant enrichment of gene deletions involving B cell differentiation, activation and proliferation, and regulation of cytokine-mediated signaling pathway at relapse (Benjamini Hochberg test, p<0.01). Table 1 summarizes the frequencies of the most retained or acquired CNA at relapse in at least 4 out of 15 patients. Besides the high genetic heterogeneity observed, some recurrent CNA could be identified such as 9p, 1q, 12q, 22q and 7p deletions and 1q, 17q, 21+ and 20+ duplications. Gene deletions in tumor suppressor genes such as TP53, FOXO1, FOXO3 or RB1 were detected in 3 patients.

Summary/Conclusions: BCP-ALL has a high genetic heterogeneity at relapse, with most of the genetic alterations playing important roles for disease progression. This heterogeneity points out the need for search of personalized treatment tools better on their molecular alterations. Finally, the Instituto de Salud Carlos III, Ministerio de Economía and Competitividad, Spain, Red Temática de Investigación Cooperativa en Cáncer (RTICC, FEDER), (RD12/00036/0044 ; Sociedad Española Hematología y Hemoterapia; 2014 SGR225 (GRE) Generalitat de Catalunya; Fundació Internacional Josep Carreras, Celgene Spain and ‘la Caixa’ Foundation.

E832

IGF1R/IRS PHARMACOLOGICAL INHIBITION REDUCES CELL PROLIFERATION AND MIGRATION IN ACUTE LYMPHOBlastic LEUKEMIA CELLS

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Background: Acute lymphoblastic leukemia (ALL) is a hematological malignancy with more than 10,000 patients-diagnosed each year in the USA. The main cause of death is due to relapse. Recent studies have suggested that LPCs (leukemia-propagating cells) are responsible for relapse. The implication of the IGF1R/IRS signaling pathway in the regulation of cell proliferation and migration in ALL cells is not well understood.

Aims: To identify the potential molecular basis of LPCs-mediated relapse, the gene expression profiles of the sorted LPCs and other cell fractions from patients with de novo Ph+ALL were compared.

Methods: Twenty patients with de novo Ph+ALL were enrolled for this study at Peking University Institute of Hematology from 2015 to 2016. The LPCs (CD34+CD38-CD58-) and other cell fractions (including CD34+CD38+CD58+ and CD34+CD38+CD58+ who were sorted from the bone marrow mononuclear cells of de novo Ph+ALL patients (N=3) using a FACs Aria II. Differential expression analysis between LPCs and the other cell fractions were performed using RNA sequencing (RNA-Seq) and the DESeq R package (1.10.1). Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. RNA-Seq results were partially validated by a TaqMan-based real-time quantitative polymerase chain reaction (qRT-PCR) technique. Moreover, cell cycle status was compared between LPCs and other cell fractions in de novo Ph+ALL patients (N=20) by flow cytometry.

Results: 1021 genes (301 up-regulated and 720 down-regulated), 1245 genes (354 up-regulated and 891 down-regulated) and 1228 genes (248 up-regulated and 980 down-regulated) were differentially expressed between LPCs and Other Cell3 (patient No 3), respectively. Most of differential expression of genes (DEGs) are related to the regulation of cell cycle and metabolism. GO analysis identified enriched terms of biological functions in DEGs including ATP binding, ribonucleotide binding process, nucleoside binding process, DNA replication, primary metabolic process, etc. KEGG analysis showed significantly enriched signaling pathways involved in DEGs including cell cycle, DNA replication, nucleotide metabolic pathways, biosynthesis of amino acids, glutathione metabolism, p53 signaling pathway, etc. Consistent with RNA-Seq results, mRNA levels of the cell cycle-related genes, such as CDK4 and HDAC1, were significantly lower in LPCs fractions than those in other cell fractions. Moreover, the frequencies of quiescent cells in LPCs were significantly higher than those in other cell fractions.

Summary/Conclusions: Distinctive gene expression profiles and cluster, which are mostly related to the regulation of cell cycle and metabolism, were demonstrated in the sorted LPCs fractions in de novo Ph+ALL patients (N=20). Therefore, our data indicate that it would be of value to develop LPCs biomarkers to contribute to personalized leukemia therapy and the need to identify therapeutic targets directed toward LPCs in Ph+ALL.

E834

T-CELL LEUKEMIA SENSITIVITY TO FARNESYL TRANSFERASE INHIBITION USING TIPIFARNIB

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Background: Relapse remains one of the major obstacles in Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ALL) even after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Relapse of Ph+ALL may result from persistence of residual progenitor cells, LPCs, which are defined by their ability to initiate human leukemia and self-renew in immunocompromised mice. In acute myeloid leukemia, higher LPCs frequencies and a gene expression profile typical of LPCs at diagnosis are predictive of unfavorable clinical outcomes. Furthermore, CDKN2A/B mutation is enriched in the CD34+CD38-CD58- fraction using a xenograft assay. Moreover, our cohort study indicate that the LPCs phenotype at diagnosis is an independent risk factor for relapse in Ph+ALL. However, little is known about the differential gene expression profiles between LPCs and the other cell fractions in de novo Ph+ALL patients.

Aims: To identify the potential molecular basis of LPCs-mediated relapse, the gene expression profiles of the sorted LPCs and other cell fractions from patients with de novo Ph+ALL were compared.

Methods: Twenty patients with de novo Ph+ALL were enrolled for this study at Peking University Institute of Hematology from 2015 to 2016. The LPCs (CD34+CD38-CD58-) and other cell fractions (including CD34+CD38+CD58+ and CD34+CD38+CD58+) were sorted from the bone marrow mononuclear cells of de novo Ph+ALL patients (N=3) using a FACs Aria II. Differential expression analysis between LPCs and the other cell fractions were performed using RNA sequencing (RNA-Seq) and the DESeq R package (1.10.1). Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. RNA-Seq results were partially validated by a TaqMan-based real-time quantitative polymerase chain reaction (qRT-PCR) technique. Moreover, cell cycle status was compared between LPCs and other cell fractions in de novo Ph+ALL patients (N=20) by flow cytometry.

Results: 1021 genes (301 up-regulated and 720 down-regulated), 1245 genes (354 up-regulated and 891 down-regulated) and 1228 genes (248 up-regulated and 980 down-regulated) were differentially expressed between LPCs and Other Cell3 (patient No 3), respectively. Most of differential expression of genes (DEGs) are related to the regulation of cell cycle and metabolism. GO analysis identified enriched terms of biological functions in DEGs including ATP binding, ribonucleotide binding process, nucleoside binding process, DNA replication, primary metabolic process, etc. KEGG analysis showed significantly enriched signaling pathways involved in DEGs including cell cycle, DNA replication, nucleotide metabolic pathways, biosynthesis of amino acids, glutathione metabolism, p53 signaling pathway, etc. Consistent with RNA-Seq results, mRNA levels of the cell cycle-related genes, such as CDK4 and HDAC1, were significantly lower in LPCs fractions than those in other cell fractions. Moreover, the frequencies of quiescent cells in LPCs were significantly higher than those in other cell fractions.

Summary/Conclusions: Distinctive gene expression profiles and cluster, which are mostly related to the regulation of cell cycle and metabolism, were demonstrated in the sorted LPCs fractions in de novo Ph+ALL patients (N=20). Therefore, our data indicate that it would be of value to develop LPCs biomarkers to contribute to personalized leukemia therapy and the need to identify therapeutic targets directed toward LPCs in Ph+ALL.
Background: T-cell leukemia is a collection of aggressive disorders with unfavorable outcome, in which targeted treatments are still at a preliminary phase. The RAS/MEK/ERK pathway is crucial for TCR signaling of T-cells and it is deregulated in T-cell acute lymphoblastic leukemia/lymphoma (T-ALL). Farnesyl transferase inhibitors (FTIs) block the localization of some RAS proteins to the intracellular membrane, thereby inhibiting their activation. Tipifarnib is a potent and specific FTI with a prominent anti-proliferative effect in some RAS mutated cells.

Aims: This study tests tipifarnib in T-cell lines for in vitro sensitivity and for biomarker discovery, both genomic and immunohistochemical.

Methods: We selected those cell lines with available genomic data from COSMIC, CCLCell or generated by our group. The MAPK, NFAT, NFKB and JAK/STAT pathways were tested by immunohistochemical analysis over FFPE-cell lines at baseline. The range of drug concentrations to perform IC50 analysis was established between 0-10,000 nM (ten points). Cell proliferation analyses were performed using CellTiter-Glo® Luminescent Cell Viability Assay kit from Promega (Madison, WI, USA), following manufacturer’s instructions at 0h, 48h and 96h. All experiments were done in sextuplet and all numerical data were expressed as the average of the values ± the standard error of the mean. IC50 analyses were performed with GraphPad Prism v5. Clinically-relevant drug sensitivity was defined as IC50 <100nM at 96h. Targeted sequencing was performed using NextSeq. Targeted analyses were performed with GraphPad Prism v5. Clinically-relevant drug sensitivity was defined as IC50 <100nM at 96h. Targeted sequencing was performed with NextSeq. IC50 and 96h. All experiments were done in sextuplet and all numerical data were expressed as the average of the values ± the standard error of the mean. IC50 analyses were performed with GraphPad Prism v5. Clinically-relevant drug sensitivity was defined as IC50 <100nM at 96h. Targeted sequencing was performed with NextSeq. IC50 and 96h. All experiments were done in sextuplet and all numerical data were expressed as the average of the values ± the standard error of the mean. IC50 analyses were performed with GraphPad Prism v5. Clinically-relevant drug sensitivity was defined as IC50 <100nM at 96h. Targeted sequencing was performed with NextSeq.

Results: 59.1% (n=13) of cell lines were sensitive to tipifarnib at concentrations which are readily achievable in the clinic (i.e. IC50 <100nM at 96h). 45.5%, 50% and 27.3% of cell lines harbored mutations in RAS, RAS-guanine nucleotide exchange factors (GEFs) and RAS-GTPase activating proteins (GAPs) genes, respectively. The mutational state of RAS-GEFs genes and NOTCH1 were associated with drug sensitivity. Strikingly, the mutualional state of NOTCH1 was associated with tipifarnib sensitivity. The activation of the MAPK pathway biomarker, ERK, was significantly associated (p=0.046) with drug sensitivity. Conversely, RelB (NFKB pathway) was associated with drug resistance (p=0.007). The same findings were observed with the presence of mutations in RAS-GEFs genes and NOTCH1 and ERK activation (p=0.015 and p=0.023) and the absence of RelB (p=0.02 and p=0.017).

Summary/Conclusions: This study shows tipifarnib as a potential therapeutic option in T-cell leukemias. The mutational state of NOTCH1 could constitute a predictor of sensitivity in T-cell leukemias. Furthermore, p-ERK and RelB could serve as potential biomarkers of tipifarnib sensitivity and resistance, respectively.

Acute lymphoblastic leukemia — Clinical

E835

HOSPITALIZATION FOR PATIENTS IN THE U.S. AND EU TREATED WITH INOTUZUMAB OZOGAMICIN VS STANDARD OF CARE FOR RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA IN A GLOBAL PHASE 3 TRIAL

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Background: Inotuzumab Ozogamicin (InO), an anti-CD22 antibody-calicheamicin conjugate, with its once a week one-hour infusion schedule, has demonstrated lower hospital utilization, in association with a clinically meaningful improvement in overall survival, high rate of complete remission, favorable patient-reported outcomes (PRO), and generally manageable safety profile versus standard of care (SOC, intensive chemotherapy) for relapsed/refractory acute lymphoblastic leukemia (R/R ALL) in the phase 3 INO-VATE trial.

Aims: This study aims to determine the regional-specific hospitalization days per patient in the INO-VATE trial.

Methods: Patients receiving study treatment (safety population) and recruited from the US and the EU were included in the analyses. The total number of days hospitalized for each patient was calculated. Hospital days prior to randomization and those after the end of study treatment were excluded. Due to different durations of treatment for InO and SOC (median 1 vs 3 cycles), calculations were reported for cycle 1 treatment period (randomization to end of cycle 1) and for the entire treatment period (all cycles - randomization to end of treatment).

Results: A total of 264 patients from the safety population of the phase 3 INO-VATE trial were available for the analyses. 149 were from the US, and 115 from 11 of the EU countries. The percentage of patients requiring hospitalization was lower for InO compared to SOC (Table). The median and mean hospitalization days were shorter for patients in the InO arm compared to the SOC arm across both regions. The difference between the two treatment arms appears to be greater in the US compared to the EU. Hospitalizations in the US appear to be shorter than in the EU, particularly for patients receiving InO.

Table 1. Hospitalizations in R/R ALL patients from the INO-VATE trial.

<table>
<thead>
<tr>
<th></th>
<th>Hospitalized (%)</th>
<th>Mean (Days)</th>
<th>Median (Days)</th>
<th>Hospitalized (%)</th>
<th>Mean (Days)</th>
<th>Median (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>115/264</td>
<td>32 (85%)</td>
<td>17 (13, 48)</td>
<td>149/264</td>
<td>40 (100%)</td>
<td>36 (30, 83)</td>
</tr>
<tr>
<td>Cycle 1</td>
<td>11/115</td>
<td>26 (88%)</td>
<td>17 (13, 48)</td>
<td>10/149</td>
<td>38 (100%)</td>
<td>36 (26, 48)</td>
</tr>
<tr>
<td>All cycles</td>
<td>25/115</td>
<td>26 (100%)</td>
<td>20 (17, 36)</td>
<td>25/149</td>
<td>39 (100%)</td>
<td>37 (31, 76)</td>
</tr>
</tbody>
</table>

Summary/Conclusions: InO treatment in R/R ALL is associated with less hospitalization across both the US and EU compared to SOC, consistent with InO’s better efficacy, tolerability, PRO and dosing schedule. The finding that US has lower hospitalization than the EU might be explained by different patient care practices in the two regions. Given that hospitalization is the biggest cost driver in cancer care, the data suggest both EU and US could benefit from cost-savings of less hospitalization with InO treatment.

E836

NON-INTENSIVE BUT NON-INTERRUPTIVE TREATMENT WITH FEWER ALLO-HSCT IS EFFECTIVE STRATEGY FOR ADULT PH-NEGATIVE B-CELL PRECURSOR (BPC-) ALL: OUTCOME OF THE RUSSIAN PROSPECTIVE MULTICENTER ALL-2009 STUDY

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Background: As Ph-negative-BCP-ALL in adults remains less favorable in prognosis than T-ALL, and by expert opinion needs intensive protocols with high proportion of allo-HSCT, the results of treatment based on the different approach need to be escalated but non-interruptive treatment with low numbers of allo-HSCT — may be of interest and can provide new insights to the common view.

Aims: to evaluate survival data and risk groups in Ph-neg-BCP-ALL pts in the RALL-study.
Methods: The ALL-2009 (NCT01193933) was initiated in Apr 2009. The treatment plan was identical for all risk groups with allo-HSCT indicated only for very high-risk BCP-ALL (t(4;11), t(1;19), WBC >100). Since Apr 2009 till Dec 2016, 329 Ph-negative ALL pts (age 28 y (15-55), f/m 147/182) were recruited. Phenotype was unknown in 6 pts, biphenotypic AL was diagnosed in ~1,2% (n=4), T-ALL/LBL in 36.7% (n=125), BCP-ALL in 59.1% (n=194). Among BCP-ALL, there were 54 early pre-B ALL (27.8%), 101 common-ALL (52%), 39 pre-B ALL (20.2%). In BCP-ALL pts m.age was 27 y (15-54), f/m 99/95, initial WBC 94,10⁹⁰⁰ (0,4-8,99,0), LDH 901 IU (31-13,059), CNS leukemia- in 17 pts (8,7%), mediastinal mass- in 3 (1,5%), splenomegaly- in 111 (57,2%). Standard cytogenetics was detected in 124 pts (64%), 11 had no mitosis, so information is available in 58,2% (n=118). 43.4% of BCP-ALL (n=49/113) pts had normal karyotype (NK); 7,9% (n=9) and 1,8% (n=2) - had t(4;11) and t(1;19) respectively; other abnormalities were detected in 53 (46,9%), including p53 (3,2%), +8 (6,3%), complex karyotype (7,9%), high hyperdiploidy (16p), delp16 (22,2%), etc. 9 BCP-ALL patients (n=7,4%) were not qualified by the risk in the data-base: 68,1% (n=126) were attributed to the high risk (HR) group (WBC ≥30, EGIL BI, LDH>2N; late CR; t(4;11)-pos). The analysis was performed in Feb 2017. 191 pts were available for induction outcomes, DFS and relapse probability (RP), and all pts – for overall survival (OS).

Results: CR rate in 191 pts was 87,4% (n=167), induction death occurred in 8,9% (n=17), resistance was registered in 3,7% (n=9). Late responders constituted 13,6% (n=26). Death in CR on chemotherapy was 6,3% (n=12) and 1 death after alloHSCT. All-HSCT was performed in 13 (6 - matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%), 11 of them – in death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (7,4%)...
E839

SINGLE-AGENT MOR208 IN PATIENTS WITH RELAPSED/REFRACTORY (R/R) B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (B-ALL): A SINGLE-ARM PHASE II STUDY

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Background: CD19 is a type I transmembrane glycoprotein that is expressed throughout B-cell development until terminal plasma cell differentiation. CD19 is also broadly and homogeneously expressed across different B-cell malignancies, including B-ALL. MOR208 is a CD19 monoclonal antibody with an enhancedFc region, which leads to a potentiation of antigen-dependent cell-mediated cytotoxicity and antigen-dependent cell-mediated phagocytosis. Aim: To evaluate the efficacy and safety of single-agent MOR208 in the treatment of patients with R/R B-ALL.

Methods: This is a single-arm phase II study of MOR208 in patients aged ≥16 years with histologically confirmed R/R B-ALL with progression after at least one prior therapy. Patients with Philadelphia-chromosome-positive (Ph+)-B ALL were excluded. A 2:1 ratio of dose-limiting toxicity (DLT) to non-dose-limiting toxicity (NDLT) was planned. The primary endpoint was the overall response rate. The trial was prematurely terminated due to insufficient evidence of single-agent activity leading to slow recruitment.

Results: 22 patients were enrolled; median age was 16 years (range 16–79); 12 (55%) patients were male; 6 (27%) patients had previously received an allogeneic stem cell transplant (SCT), the most common disease subtype was pre-B-ALL (15; 68%) and 2 (9%) patients had Ph+ B-ALL. 6 (27%) patients received ≥2 cycles of MOR208 and had a subsequent response assessment. Responses were seen in 2 patients; and included a CR and a CRi, giving an overall response rate of 9%. These 2 patients received extended MOR208 treatment. A further 3 (14%) patients did not fulfill the criteria for PR but did not progress; 16 (73%) patients withdrew before completing cycle 2, in most cases due to progressive disease (PD). The patient in CR met the criteria for allo- genetic SCT, but declined this at the time; response duration was 6 weeks, with subsequent PD. The patient with the CRi had a response duration of at least 4 weeks, but discontinued due to a treatment-emergent adverse event (TEAE), sclerodingiosis. For 12 out of 13 patients with available data, MOR208 treatment led to a rapid reduction in blast/B-cell counts in the peripheral blood; in most cases a reduction of >90% within 1 week of treatment initiation was seen. In patients with a prior marrow response, ≥1% of all-inclusive TEAEs were febrile neutropenia, thrombocytopenia, neutropenia, sepsis and hyperglycemia (each 5 [23%] patients). Infusion-related reactions were reported in 13 (59%) patients; all occurred on day 1 of cycle 1 and were mostly grade 1 or 2, with one grade 3 event; all patients recovered on the same day. Pharmacokinetic data were comparable with previous clinical studies and anti-MOR208 antibodies were not detected.

Summary/Conclusions: MOR208 showed signs of clinical efficacy with rapid reductions in peripheral blood blasts in most patients with R/R B-ALL, but the durability and frequency of achieving CRs was suboptimal, which was not unex- pected for a single-agent monovalent antibody. The results are consistent with the durability and frequency of achieving CRs was suboptimal, which was not unex- pected for a single-agent monovalent antibody. The results are consistent with the MOR208 with previously studies and favorable, further development as a part of a combination treatment in R/R B-ALL remains a promising approach.
heavy chain (IgVH, N=62) or T-cell receptor (TCR, N=3) clonal rearrangements was carried out by 8-color FCM (N=73) and RQ-PCR of immunoglobulin.

Results:
Figure 1.

Figure 1.

Results: Total number of 110 patients was evaluated. Nine of them (8.7%) who did not reach a hematological remission on D26 were excluded from the study. The Kaplan-Meier plot of the final cohort was generated. MRD evaluation was carried out by 8-color FCM (N=73) and RQ-PCR of immunoglobulin heavy chain (IgVH, N=62) or T-cell receptor (TCR, N=3) clonal rearrangements and BCR-ABL (N=24), MLL-AF4 (N=4) and E2A-PBX1 (N=1) fusion genes. Methods with strongest sensitivity for OS prediction on D26 were RQ-PCR with 1.0×10^{-3} cut-off (4-year OS: 76.6% vs 48.8%, median OS: not reached vs 39.1 months; p=0.012) and FCM (4-year OS: 78.3% vs 30.3%; median OS: not reached vs 27.4 months; p=0.016). The most sensitive method in W11 was RQ-PCR with every positive result considered MRD positive (4-year OS: 79.6% vs 53.1%; median OS: not reached vs 46.5 months; p=0.013). Flow cytometry and PCR with other cut-offs were not sufficiently sensitive. The sub-analysis of Ph-negative patients has shown the same results for RQ-PCR (p<0.01).

Summary/Conclusions: We have analyzed both RQ-PCR and FCM to be suitable methods for MRD assessment on D26 of induction in adult ALL patients receiving an intensive treatment. Furthermore, it seems convenient to take any RQ-PCR positivity (even below 1.0×10^{-4}) into account in W11 and later stages of treatment. FCN can be used for MRD assessment on D26, but it is not sufficiently sensitive in later stages of treatment. We suggest using RQ-PCR as a method of choice for MRD assessment in adult ALL while retaining FCM as a backup method for patients without applicable RQ-PCR target or when faster MRD evaluation is needed.

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E842
QUALITY-ADJUSTED LIFE YEARS (QALY) FOR INOTUTUMAB OZOGAMICIN VS STANDARD OF CARE FOR RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA (R/R ALL)
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Background: Inotuzumab Ozogamicin (InO), an anti-CD22 antibody-calciumeamicin conjugate, has demonstrated superior clinical activity versus standard of care (SOC; intensive chemotherapy), including clinically meaningful improvement in overall survival (OS), high rates of complete remission (CR) and potentially curative hematopoietic stem cell transplantation (HSCT), and favorable patient-reported outcomes for R/R ALL in the phase 3 InO-VATE trial. Quality of life (QoL) is an important consideration for R/R ALL patients in both short- and long-term survival.

Aims: This study aimed to estimate mean overall survival adjusted for QoL (QALY) for patients treated with InO vs SOC.

Methods: A Markov model was developed with five health states - No CR, CR, post-HSCT, progression, and death. Lengths and transition probabilities between health states and mortality rates were based on the InO-VATE trial. These rates were extrapolated to a lifetime horizon using parametric survival curves fitted to available OS data, and published literature for survival beyond available data. Utilities (QoL valuations) for each health state were based on the patient-reported EQ-5D scores collected in the InO-VATE trial and a literature review for health states not captured in the trial. Disutilities from adverse events experienced during and after treatments, including adverse events as a result of subsequent HSCT such as veno-occlusive disease (VOD), were taken into account in overall QoL. Outcomes were discounted at 1.5% and half-cycle corrected.

Results: The estimated mean LY and QALY in each health state for InO and SOC and their differences are shown in Table. Most gains in LY and QALY for InO vs SOC were from InO and HSCT. The difference in LY and QALY between the two therapies is larger in the InO arm as more patients achieved a CR and could undergo a HSCT. Additionally, a "tail-of-the-curve" survival gain Post-HSCT is observed in InO but not SOC.

Table 1.

<table>
<thead>
<tr>
<th>Health state</th>
<th>InO</th>
<th>SOC</th>
<th>InO-SOC</th>
<th>QALY</th>
<th>SOC-QALY</th>
</tr>
</thead>
<tbody>
<tr>
<td>No CR</td>
<td>0.07</td>
<td>0.13</td>
<td>-0.06</td>
<td>0.05</td>
<td>0.08</td>
</tr>
<tr>
<td>CR</td>
<td>0.25</td>
<td>0.06</td>
<td>0.19</td>
<td>0.06</td>
<td>0.07</td>
</tr>
<tr>
<td>Post HSCT</td>
<td>2.58</td>
<td>2.62</td>
<td>-0.04</td>
<td>2.20</td>
<td>0.44</td>
</tr>
<tr>
<td>Progression</td>
<td>0.16</td>
<td>0.12</td>
<td>0.04</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>Total</td>
<td>3.48</td>
<td>3.77</td>
<td>-0.29</td>
<td>2.48</td>
<td>0.67</td>
</tr>
</tbody>
</table>

*Increment values may not always correspond to differences between LYs and QALYs due to rounding.

Summary/Conclusions: This analysis taking into account both quantity and quality of life estimates shows that InO offers an average of nearly 2 more years of QALY compared to SOC in R/R ALL, based on higher CR and HSCT rates, "tail-of-the-curve" survival gains, and better QoL. This can help inform patients, physicians and payers in decision making.

E843
A COST-EFFECTIVE, HIGH SENSITIVITY 10-COLOR SINGLE TUBE FLOW-CYTOMETRY BASED B-CLL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA MINIMAL RESIDUAL DISEASE (MRD) ASSAY WITH STUDY OF ARTIFACTS AND MIMICS
G. Chatterjee1,*, D. Dhaliwal1, S. Ghogale1, B. Yajamanam1, N. Deshpande1,

....
FC-MRD assay with high sensitivity of at least 1 in 10⁵ and applicability in >97% P. Tembhare

We studied 230 BCPALL MRD samples. FC-immunophenotyping and describe their prevalence and immunophenotypic features.

Aims: 1. To study the applicability and sensitivity of a 10-color high event single tube FC-MRD assay for BCPALL and describe the frequency and immunophenotypic features. 2. To document the rare BM cellular elements and artifacts causing interference in analysis.

Methods: We studied 230 BCPALL MRD samples. FC-immunophenotyping was performed on Navios flow-cytometer using bulk-lysis-and-stain method and data was analyzed with Kaluza-software. MRD was monitored using 10-color single tube FC-MRD assay including CD45, CD10, CD19, CD20, CD34, CD38, CD58, CD98, CD123 and CD25/CD73 with an additional 4-color nuclear dye (SYTO13) tube. Samples with cluster of ≥20 and ≥2 leukemia associated phenotypes (LAIPs) were called MRD-positive. High number of events were acquired for MRD-assay (1.5 to 6 million). To evaluate the applicability of assay, number of LAIPs were determined in diagnostic and MRD samples. In addition, the frequency and antigen expression pattern of mimics and artifacts were studied.

Results: We studied 230 BCPALL MRD samples. High number of events was acquired for MRD-assay with median-events 3427000 (range, 1678000 to 6052800). We determined the limit of detection (LOD=10 events) and limit of quantification (LQ=0.01% to 0.003%) by performing the following assays. MRD was positive in 107 (46.5%) samples with median of 0.135% and range of 0.0003% to 48.3%. We categorized positive MRD results into samples with MRD <0.001%, 0.001% - <0.01%, 0.01% - <0.1%, 0.1% - <1.0% and >1% and they were respectively 1.74%, 10.43%, 13.48%, 5.65% and 10.00%. Furthermore, in 24 samples with MRD-positive ≤0.01% and >1.5 million acquired-events, the results were compared between time-gated initial 500000-events and >1.5 million acquired-events and all events acquired. Sixteen samples among these were found to be negative in initial 500000-events and eight in initial 1000000-events highlighting the importance of acquisition of >1.5 million cells. Further, we categorized different rare cellular events and artifacts in the following way: 1) CD34+ mature B cells; 2) CD10+ mature B cells; 3) CD73+ mesenchymal/stem cells and endothelial cells; 4) CD123+ CD19+ 7PD precursors; 5) CD86+ CD58+B cell precursors (BCP); 6) CD19+ NK cells (Table 1). We also described their immunophenotypic features highlighting the differentiating features from MRD and B cell precursors (Figure 1).

Figure 1.

Summary/Conclusions: We established a cost-effective 10 color single tube FC-MRD assay with high sensitivity of at least 1 in 10⁵ and applicability in >97% BCPALL MRD samples. We also described the frequency and extent of different cellular events and artifacts that can interfere with high-sensitivity BCPALL FC-MRD analysis. The knowledge regarding presence and antigen expression pattern of these cellular events and artifacts are critical to avoid potential false positive results.
with b-blockers, as they could limit antihypertensive toxicity by their heart rate-lowering activity and antioxidant effect. All the 8 patients subsequently improved in both GLS and LVEF values, despite the occurrence of one episode of mild hypotension in 2 patients.

**Summary/Conclusions:** All children, even if exposed to low doses of antihypertensive, show early signs of LV impairment. Overt drop in LVEF, when present, mostly follow GLS alterations. Alterations seem more frequent in HR pts, possibly due to the higher burden of both leukemia itself and HR treatment. Further studies on wider series are needed to confirm the relevance of the early diagnosis of LV preclinical dysfunction in pediatric ALL patients.

**E845**

**NUDT15 VARIANT CAUSING HEMATOPOIETIC TOXICITY WITH LOW 6-TGL LEVEL IN KOREAN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA**

H. Hoe Ko1*, E. Sang Yi2, Y. Bae Choi3, N. Hee Lee4, J. Won Lee4, K. Hee Yoo1, K. Woong Sung1, R. Choi2, S.-Y. Lee1

1Pediatrics, Sungkyunkwan Univ School of Medicine, Samsung Medical Center, 2Department of Pediatrics, Korea University Guro Hospital, Korea University College of Medicine, 3Department of Pediatrics, Chung-Ang University Hospital, Seoul, 4Department of Pediatrics, Cha Bundang Medical Centre, Cha University, Seongnam, 5Department of Laboratory Medicine and Genetics, Sungkyunkwan Univ School of Medicine, Samsung Medical Center, Seoul, Korea, Republic Of

**Background:** NUDT15 polymorphism has been recently identified as a determinant of thiopurine intolerance. 6-thioguanine nucleotides (6-TGN) is monitored to prevent hematopoietic toxicity in acute lymphoblastic leukemia (ALL).

**Aims:** This study intended to evaluate the impact of NUDT15 polymorphism on thiopurine intolerance and 6-TGN level in Korean children with ALL.

**Methods:** Genotyping of NUDT15 was performed in 258 children with ALL who were registered in Samsung Medical Center. According to NUDT15 diplotype, patients were classified into low risk (LR, wild-type), intermediate risk (IR, heterozygous information), or high risk (HR, homzygous or compound heterozygous variant). Total of 182 were finally included after 76 patients were excluded for TPM7 variation or lack of information during maintenance therapy; LR (n=131), IR (n=46), and HR (n=5).

**Results:** The least 6-mercaptopurine (6-MP) dose (mg/m2/day) was administrated on day 1 (8.9±1.9), day 15 (9.5±1.9), LR 31.8±7.1, IR 33.8±7.1, HR 38.3±7.5. LVEF decreased on the longest days of therapy interruption (HR 167 vs IR 30 vs 15, p<0.01) and days of leukopenia (HR 131 vs IR 92 vs LR 59, p<0.01). The lowest WBC and platelet counts and hemoglobin level were observed in HR. 6-TGN level (pmole/8x10^18 RBC) divided by 6-MP dose (mg/m2) was the lowest in HR group (HR 4.4 vs IR 13.3 vs HR 14.7, p<0.01).

**Summary/Conclusions:** Patients with NUDT15 variants encountered significant thiopurine intolerance even with low level of 6-TGN. This concurs with the existing hypothesis that NUDT15 protein may prevent incorporation of thiopurine active metabolites into DNA. Therefore 6-TGN monitoring is not useful to predict hematopoietic toxicity for patients with NUDT15 variant.

**E846**

**USING NEXT GENERATION SEQUENCING TO DETECT CLONAL TRG AND TRB GENE RARRANGEMENTS**

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1Invivoscribe Technologies, Inc., San Diego, United States

**Background:** During early T-cell development, somatic rearrangements occur within T cell receptor beta (TRB) locus that bring together, sequentially, the V-(D-)J gene segments of TRG, TRB, and TRG+ regions (J) are the current gold standard for clonality testing in suspected B-cell malignancies. Recently, next-generation sequencing (NGS) based approaches have been sequenced for immune receptor genes have been developed that improve sensitivity and identify the specific V-(D-) J DNA sequences required to track clones in follow-up testing. We developed comprehensive LymphoTrack® MiSeq and PCR-CE assays for TRB and TRB demonstrated good concordance.

**Methods:** Rearranged products from within the TRG and TRB loci were generated by PCR using proprietary multiplex master mixes with consensus primers targeting all TRG and TRB V and J exon families, synthesized with MiSeq specific adaptor and individual barcode ID sequences. The PCR products were purified, quantified and pooled into equimolar library. The final library was sequenced on the MiSeq. The sequencing data FASTAQ output file was analyzed using Invivoscribe’s LymphoTrack® software. The software generated frequency distributions for the top 200 rearranged sequences, identified the DNA sequences, generated V-J assignments and V-J usage. Cell line DNA known TRG and TRB V-J rearrangements was tested for the analytical performance. DNA from different clinical sample type (FFPE, PB, and BM) was used to assess the clinical performance.

**Results:** This NGS assay was able to correctly detect all known TRB and TRG rearrangements from cell line DNA. The on-target reads per sample were 90% - 100%. Excellent linearity (R²>0.90), sensitivity of 2.5% for clonality, and reproducibility (<20% CV) were demonstrated with serial dilutions of contrived cell line DNA. The clinical performance of the LymphoTrack® TRG + TRB NGS assays was evaluated on different clinical samples that have also been tested using the PCR-CE TRG and TRB assays. Assessment of clonality using the LymphoTrack® MiSeq and PCR-CE assays for TRG and TRB demonstrated good concordance.

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**Summary/Conclusions:** This combo NGS assay provides a fast, simple, and accurate method to detect clonality. In combination with the LymphoTrack software, the TRG + TRB MiSeq assay can identify clonal TRG and TRB V-(D) J rearrangements and the specific V-(D) J region DNA sequences required to track clones in follow-up testing. Excellent concordance of clonality with specific rearrangements was demonstrated between LymphoTrack® MiSeq and PCR-CE method.

**Background:** PCR-based capillary electrophoresis (PCR-CE) methods target the V-(D)-J region of the IgM and IgG heavy chain (IGH) framework 1, 2, 3 (FR1, FR2, FR3), and joining regions (J) are the current gold standard for clonality testing in suspected B-cell malignancies. Recently, next-generation sequencing (NGS) based approaches have been sequenced for immune receptor genes have been developed that improve sensitivity and identify the specific V-(D)-J DNA sequences required to track clones in follow-up testing. We developed comprehensive LymphoTrack® IGH (FR1, FR2, FR3) Assays for both the illumina® MiSeq® and Thermofisher Scientific® Ion PGM™ platforms, which detect the vast majority of rearrangements in a single NGS run. In this pilot study, we compared the performance of both LymphoTrack® IGH MiSeq and PGM Assays to the IGH PCR-CE assay by testing in 59 anonymized, blinded clinical samples.

**Aims:** To assess the clinical performance of LymphoTrack® IGH MiSeq and PGM Assays.

**Methods:** LymphoTrack® IGH Assay has been developed for both the MiSeq and PGM platforms. Proprietary consensus primers targeting the V and gene segments of IGH were designed to include both platform specific adapter sequences and individual barcodes so multiple independent PCR products could be combined and sequenced together on the MiSeq or PGM platforms. MiSeq IGH FR master mixes were individually manufactured with 24 indices to allow analysis of 22 samples with 2 controls. PGM IGH FR master mixes were manufactured with 12 indices to allow analysis of 10 samples with 2 controls. DNA was extracted from 21 PB, 37 FFPE and 1 BM clinical samples. Single step PCR amplification of 50 ng DNA input was followed by amplicon purification. Equimolar amounts of purified amplicons were pooled and loaded onto the Ion Torrent PGM instrument using LymphoTrack® IGH NGS assays was evaluated on 59 clinical samples that have also been tested using the PCR-CE IGH assay. Only samples that met the specimen and data acceptance criteria for both methods were evaluated to determine concordance. Assessment of clonality using the LymphoTrack® IGH MiSeq and PGM-CE assays demonstrated good concordance. The clinical performance was then validated using the LymphoTrack® IGH MiSeq and PCR-CE assays. Concordance in clonality calls between the LymphoTrack® IGH MiSeq and MiSeq was 100% (51/51).

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**Aims:** To assess the clinical performance of LymphoTrack® IGH MiSeq and PGM Assays.

**Methods:** LymphoTrack® IGH Assay has been developed for both the MiSeq and PGM platforms. Proprietary consensus primers targeting the V and gene segments of IGH were designed to include both platform specific adapter sequences and individual barcodes so multiple independent PCR products could be combined and sequenced together on the MiSeq or PGM platforms. MiSeq IGH FR master mixes were individually manufactured with 24 indices to allow analysis of 22 samples with 2 controls. PGM IGH FR master mixes were manufactured with 12 indices to allow analysis of 10 samples with 2 controls. DNA was extracted from 21 PB, 37 FFPE and 1 BM clinical samples. Single step PCR amplification of 50 ng DNA input was followed by amplicon purification. Equimolar amounts of purified amplicons were pooled and loaded onto the Ion Torrent PGM instrument using LymphoTrack® IGH NGS assays was evaluated on 59 clinical samples that have also been tested using the PCR-CE IGH assay. Only samples that met the specimen and data acceptance criteria for both methods were evaluated to determine concordance. Assessment of clonality using the LymphoTrack® IGH MiSeq and PGM-CE assays demonstrated good concordance. The clinical performance was then validated using the LymphoTrack® IGH MiSeq and PCR-CE assays. Concordance in clonality calls between the LymphoTrack® IGH MiSeq and MiSeq was 100% (51/51).
Summary/Conclusions: Comprehensive IGH Assays have been developed for both MiSeq and PGM platforms. These assays identify clonal IGH V-J rearrangements and provide the clonal DNA sequences of the tumor-specific clonotypes required to perform follow up testing to detect residual disease. Combining FR1, FR2 and FR3 improved the overall clonality detection rate to 96%. Both NGS-based IGH assays have demonstrated excellent concordance in detecting clonality regardless of whether clonality was determined using a PCR-CE method or with assays formatted for the MiSeq and PGM platforms.

E848
CORRELATION BETWEEN A 10-COLOR FLOW CYTOMETRIC MINIMAL RESIDUAL DISEASE (MRD) ANALYSIS AND MOLECULAR MRD IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA
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Background: Minimal residual disease (MRD) monitoring in Acute Lymphoblastic Leukemia (ALL) is an accepted standard of care in both adult and pediatric patients as one of the strongest predictive factors for disease outcome and as a stratification tool for treatment intensification and allogeneic stem cell transplant. The currently accepted standard of molecular monitoring with either immunoglobulin heavy or kappa chain (IG) or T-cell receptor (TCR) quantitative PCR (qPCR) in Philadelphia negative ALL allows for sensitive monitoring of MRD, but requires a high degree of expertise, and factors such as cost and turnaround time may limit generalized applicability of this technique. Flow cytometric MRD monitoring is utilized in many centers, with increased sensitivity seen with implementation of multi-parameter flow cytometry at 8-colours or more.

Aims: We sought to compare a 10-color flow cytometry assay for detecting MRD in B-ALL with standard molecular monitoring.

Methods: To facilitate rapid identification of MRD in patients with B-ALL, we developed a 10-colour single tube flow cytometry assay utilizing CD19, CD22, CD45, CD38, CD59, CD11c, CD10, CD45 and CD43 as markers. These markers were selected to provide at least two targets for identification of B-lineage cells, and to include the most frequently aberrant markers in precur- sor B-lineage ALL. Samples were subject to bulk ammonium chloride lysis to maximize cell yields with a target of 1 x 10^6 events. Once normal maturation patterns were established, patient samples were analyzed in parallel to standard of care molecular monitoring with either IGH/TCR qPCR in Philadelphia negative (Ph-) disease and BCR-ABL qRT-PCR in Philadelphia positive (Ph+) disease. Statistical correlation was performed in Graphpad Prism version 7.0 for linear regression and calculation of correlation co-efficient.

Figure 1.

Results: 33 samples at different time points from 13 patients were analyzed by flow cytometry. 9 samples from 9 patients were taken at diagnosis. Whilst an informative MRD phenotype was identified by flow cytometry in all 9 patients, a molecular assay was not able to be developed in one patient due to lack of an identifiable marker. 24 samples from 13 patients were tested for MRD by flow cytometry. The median lower limit of detection was 0.0078% (range 0.0016% to 0.028%) with a median lower limit of quantification of 0.018% (range 0.002% to 0.07%). A sensitivity of <0.01% was attained in 21 of 24 samples (88%). 20 samples from 11 patients were tested concurrently for MRD by both molecular and flow cytometric methods. 11 samples were in Ph- disease and 9 were in Ph+ disease. MRD was detected by both molecular and flow cytometry in 11 samples and not detected by both methods in 8 samples. In one sample, MRD was detected only by molecular at an unquantifiable level. There was a strong correlation co-efficient between molecular and flow cyto- metric MRD analysis (R²=0.905, p<0.001). Correlation was strong with both IGH/TCR based molecular analysis (R²=0.949, p<0.001) and BCR-ABL based molecular assays (R²=0.993, p<0.001).

Summary/Conclusions: 10-color flow cytometric minimal residual disease analysis with bulk lysis attains a high degree of sensitivity in minimal residual disease determination in precursor B-lineage Acute Lymphoblastic Leukemia. There was a strong correlation with molecular MRD monitoring for both quan- tification of MRD and determination of MRD negative status. Flow cytometric methods may also permit MRD monitoring in patients where a suitable molec- ular assay cannot be developed.

E849
HYPOGLYCEMIC EVENTS DURING TREATMENT OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA: OBSERVATIONS FROM TRIAL AIEOP-BFM ALL 2009
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Background: Hypoglycemia has been reported as a rare side effect in children and adolescents treated for acute lymphoblastic leukemia (ALL). It has been associated to purine nucleoside analogues (PNA), but potential relationship with asparaginase has also been described. Despite these reports, clinicians’ awareness of this risk seems to be limited.

Aims: Descriptive evaluation of symptomatic hypoglycemic events during ALL treatment.

Methods: Hypoglycemic events were analyzed among 3293 patients treated in the trial AIEOP-BFM ALL 2009 in four of the participating countries (Germany, Switzerland, Czech Republic, and Australia) between 06/2010 and 08/2016. PNA were administered during induction-consolidation, the second part of the reintensification phase (reinduction-consolidation) and during maintenance (MT). Pegylated asparaginase (PEG-ASP) was given in induction-consolidation of reintensification, as well as high-risk blocks. Additionally, the benefit of intensi- fied PEG-ASP was tested during induction-consolidation in the high-risk group, and during reinduction-consolidation/MT in the medium-risk group. Adverse events were generally captured in a targeted approach by means of defined events assessed as clinically relevant, not including hypoglycemia. Thus, data collection of these events was based on proactive reporting by the investigators. For analysis, clinical severity of the events was retrospectively graded according to patients’ capacity of action and reaction.

Results: In total, 28 hypoglycemic events were reported in 26 of the 3293 patients. 25 events in 23 patients were described as symptomatic, to which further analysis was restricted (22 precursor B- and one T-ALL; 8 standard-risk, 12 medium-risk, and 3 high-risk). Age of patients ranged between 1.7 and 15.5 years at occurrence of symptomatic hypoglycemia. Balanced ratio between both sexes can be observed (13 male, 10 female), median age was essentially similar (male 3.2 y, female 4.1 y). Hypoglycemic events occurred in induction treatment (n=1), induction-consolidation (n=4), reinduction-consoli- dation (n=4; one in standard reinduction, 3 in reinduction with intensified PEG- ASP treatment), high-risk block (n=1), and in MT (n=11; 4 events during standard MT, 6 events during MT with intensified PEG-ASP treatment, and one event 4 weeks after last PEG-ASP during MT). Seven events were reported with mild symptoms, 6 patients showed moderate symptoms, and in 12 events patients showed severe symptoms (loss of consciousness, seizure-like).

Summary/Conclusions: In accordance with previous reports, hypoglycemic events accumulated in PNA containing treatment phases, but not exclusively. Considering that 324 patients of the total cohort were treated with intensified PEG-ASP in reinduction-consolidation/MT, an additive effect of PEG-ASP and Asparaginase on a hypoglycemic metabolic condition may be assumed although a similar effect was not seen in induction-consolidation with intensified PEG-ASP. However, numbers are small and reporting bias of the present data is probable, as hypoglycemic events were not captured systematically. Inves- tigators’ attention to adverse reactions and proactive reporting might be higher.
in experimental arms as well as in case of preceding hypoglycemic events in other patients of the respective trial center. Despite these analytical limitations, our data suggest that hypoglycemia during ALL treatment is a relevant and probably underestimated clinical problem. Further investigation including possible identification of predisposing metabolic conditions is required to avoid harm to patients by this preventable complication.

**E850**

NUDT15 VARIANT IN KOREAN CHILDREN WITH ACUTE LYMPHOBlastic LEUKEMIA


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**Background:** Acute lymphoblastic leukemia (ALL) is the most prevalent pedi
ciatric cancer with cure rates approaching 90% with current therapy. Patient with ALL require long-term maintenance therapy. The combination of weekly methotrexate and daily 6-mercaptopurine (6-MP) consisted with the backbone of ALL maintenance regimens. Genetic polymorphism in thiopurine methyl
transferase (TPMT) is well known to affect the 6-MP tolerance. However preva
nence of non-function variant of TPMT is rare in Far East. Recently, a study has identified a variant of the NUDT15 gene associated with intolerance of 6-MP.

**Aims:** We examined the association between NUDT15 polymorphism and clinical data of Korean pediatric ALL.

**Methods:** NUDT15 genotyping and collection of clinical data was performed for 74 Korean pediatric ALL patients from two different hospital. For NUDT15 genotyping, DNA was extracted from whole blood/or bone marrow sample and Sanger sequencing was performed for exon 1 and 3 of NUDT15 gene.

**Results:** We found two kinds of variants, c.55_56insGAGTCG(rs869320766) in exon 1 from 8 patients and c.415C>T(rs116855232) in exon 3 from 14 patients. Of them, 7 patients had both variants and all variants were heterozy
gote. Patients could be divided to four distinct groups according to combinations of genotype (Table 1). 6-MP dose intensity in wild type was higher than three other genotypes during maintenance therapy (p=0.003) (Fig 1). The number of hospitalized days in wild type is small compared to other three genotypes (p=0.017). Frequency of febrile neutropenia, hepatotoxicity, cumulative days of antibiotic use and overall survival did not significantly differ by NUDT15 genotype.

**Table 1. Treatment outcome of children with acute lymphoblastic leukemia according to NUDT15 genotypes.**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patient No.</th>
<th>Relapse</th>
<th>Admission day during maintenance (Mon)</th>
<th>Суperm EV3 (%)</th>
<th>Суperm EV6 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild type</td>
<td>26</td>
<td>9 (34.6%)</td>
<td>13(2-245)</td>
<td>98.6±0.25</td>
<td>98.2±0.5</td>
</tr>
<tr>
<td>c.55_56insGAGTCG</td>
<td>8</td>
<td>1 (12.5%)</td>
<td>78.3±248</td>
<td>95.7±11.7</td>
<td>100.00</td>
</tr>
<tr>
<td>c.55_56insGAGTCG</td>
<td>8</td>
<td>1 (12.5%)</td>
<td>198.0±335</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>c.415C&gt;T</td>
<td>108</td>
<td>20 (18.5%)</td>
<td>198.0±335</td>
<td>98.2±0.25</td>
<td>98.2±0.5</td>
</tr>
</tbody>
</table>

**Summary/Conclusions:** Genotyping of NUDT15 could be beneficial to predict the tolerable dose of 6-MP of pediatric ALL patients.

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**E852**

TREATMENT OUTCOME OF ACUTE LYMPHOBLASTIC LEUKEMIA IN KOREAN ADOLESCENTS AND YOUNG ADULTS


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**Background:** The outcome of acute lymphoblastic leukemia (ALL) has marked
dly improved for last centuries, but the improvement was mainly observed in children under 10 years old. In contrast, the treatment outcomes of ALL in ado
dlescents and young adults (AYA) still lag beyond those of younger children.

**Aims:** We conducted this study to investigate the treatment outcome of AYA ALL in Korea, and to define any patterns of care related to the treatment out
come of AYA ALL.

**Methods:** Clinical data of 10-29 years old ALL patients diagnosed between 2002 and 2010 were extracted from Korean national health insurance service. Data about patients’ diagnosis, age, gender, mainly treated department (internal medicine vs pediatrics), usage data of medications (L-asparaginase, 6-mer
captopurine, vincristine, prednisolone or dexamethasone), hematopoietic stem cell transplantation (HSCT), radiotherapy, survival, and follow-up duration were collected. Patients who were treated with steroid over 2 weeks, and L-asparag
inase at least once in initial 2 months were considered to be treated as pediatric protocol, and who did not fulfill this criteria were considered to be treated as adult protocol.

**Results:** Total 1,223 ALL AYA patients were diagnosed between the 2002 and 2010, and excluding those who never treated, 1,208 patients underwent ALL treatment. Among them, 665 (55%) patients were treated with pediatric protocol, and the other 543 (45%) patients were treated with adult protocol. Radiotherapy was done in 278 (41.8%) and 186 (34.3%) in each group, and HSCT was done in 205 patients (30.8%) and 216 patients (39.8%) in each group, respectively. Pediatric protocol group showed significantly better overall survival compared to adult protocol group in total age (65% vs 43%, P<0.0001), 10-14 years old (76% vs 57%, P<0.0001), and 20-24 year old patients (51% vs 31%, P=0.0116). In unfavorable analysis, patient age (younger), treatment protocol (pediatric), L-
Asparaginase, 6-mercaptopurine, and steroid over 2weeks in initial 2 months were associated with better overall survival (P=0.0001 for each).

**Summary/Conclusions:** The overall survival rates in Korean AYA ALL were comparable with previous studies done at other countries. Patients treated with pediatric protocol tended to result better overall survival rate when compared to patients treated with adult protocol. Radiotherapy and early HSCT were wide
lly used in the 2000s, and further study is needed to follow up the recent trend of treatment, and outcome as a result.

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**E853**

AUTOLOGOUS TRANSPANTATION AS TIME-DEPENDENT FACTOR FOR SURVIVAL OF PATIENTS WITH T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA: STUDY DATA AND SIMULATION MODEL

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**Background:** The role of autologous hematopoietic stem cells transplantation (aHSCT) for patients with T-cell ALL is still being discussed. The resent Russia study of ALL shows the promising effect of aHSCT but there is a skepticism as the study was not randomized. The possible bias was referred to the “time selection” factor.

**Aims:** It’s need to prove that time selection can not explain the magnitude of the effect of aHSCT on patient’s survival.

**Methods:** We have developed SAS macros time-depend graphical and analytic procedures for time dependent factors: Land Mark (LM) methods, Mantel-Bay
test, Cox regression model (CM) and also a base for simulation all end points and study events like remission, transplantation, relapse and death are well approximated by a mixture of exponent distributions. Non-constant (dropping) hazard rate exists in real study data. The consequence of violation of constant hazard assumption as most possible source of biases was tested on our simu
lation model in different situations. Real data multicenter study of ALL was used to fit simulation model parameters. Russian ALL study group held a prospective multicenter trial RALL-2009 in the treatment of Ph-negative adult ALL patients based on non-intensive but non-interruptive treatment (NCT01193933). The therapy was unified for all Ph-negative ALL pts, but in T-
cell ALL/LBL autologous hematopoietic stem cell transplantation (auto-HSCT) after non-myeloablative BEAM conditioning was scheduled as late intensifica
tion (3±4 mo of CR) followed by prolonged 2 years maintenance. From Jan 2009 till Jul 2016, 30 centers enrolled 107 T-ALL/LBL pts. Median age was 28 years (15-54 y), 34 f / 73 m; early T-cell (T/I) phenotype was verified in 56

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**E851**

Abstract withdrawn.
(52.3%), mature (T-IV) - in 10 (4.0%), thymic (TII, CD1a+) ALL - in 41 pts (38.3%). T-lymphoblastic lymphoma (T-LBL - <25% b/m blasts) was diagnosed in 22 pts (20.5%). Autologous HSCT was performed in 35, allogeneic in 7 pts.

Results: The survival analysis of real data shows 4-fold dropping hazard rate. The effect of aHSCT was confirmed by LM analysis, Mantel-Bayr test - PMB=0.004, Cox model output: 1/HR=15.9, P=0.008. (Fig. 1). Simulation model for remission consists of 3 fractions: early (a=10%, r=0.05 m, δ=0.2 m), normal (a=57%, r=0.28 m, δ=1 m) and late remission (a=33%, r=1.31 m, δ=2.2 m), for survival consists of 2 fractions: short life (a=59%, r=22 m), long life (a=41%, r=600 m). (Fig.2). The first simulation experiment was performed in preposition that transplantation has no effect (HR=1). To exclude the random effect the sample size was N=4000, Mantel-Bayr and Cox model show significant (PMB=.50, P_CMR=.50, HR=.93) but LM plot demonstrates recognizable bias in transplanted patient group (Fig.3). The second experiment supposed that the existed effect of aHSCT (HR=.05), N=500. Mantel-Bayr and Cox model would show significance, but hazard ratio was underestimated (PMB=0.03, PCM=0.03, HR=.70 (0.50-0.97)). More experiments were done for repeated simulation, which demonstrated a very good agreement of Mantel-Bayr and Cox methods and their robustness.

E854 INDUCTION WITH TYROSINE KINASE INHIBITORS, CONSOLIDATION WITH FLUDARABINE, ARA-C AND DAUNOXOMEB FOLLOWED BY ALLOGENIC STEM CELL TRANSPLANTATION IS AN EFFECTIVE AND FEASIBLE STRATEGY FOR PH+ ALL PATIENTS

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Background: The prognosis of Philadelphia positive (Ph+) acute lymphoblastic leukemia (ALL) patients has improved since the introduction of tyrosine kinase inhibitors (TKI). Following TKIs treatment almost all patients rapidly achieve complete hematologic remission (CR). However, only a minority of patients obtain complete molecular response and most of all will eventually relapse without further treatment.

Aims: To determine the clinical significance of MRD on day 7 of initial steroid treatment in patients with childhood ALL we analyzed data from 173 patients treated with modified St Jude Total XV therapy between 1 January 2008 and 31 December 2015.

Methods: According to our previous successfull results with high dose methypredniosolone (HDMPX) we add 7 days of HDMPX to the modified St Jude Total XV as an initial treatment and randomized patients at doses of 10mg/kg/d or 20mg/kg/d HDMPX: not exceeding at maximum 1000mg methylpredniosone. After the end of 7th day of steroid concomitant chemotherapy was given and the doses were tapered gradually to 5mg/kg/d and 10mg/kg/d in each group respectively. By the 3rd week of treatment steroid dose was tapered to 2mg/kg/d in both groups and continued with this dose till the end of 3rd week of induction phase. MRD levels were studied at the 15th, 22nd and 42nd days of induction according to the protocol. However, we also analysed steroid response rate by the peripheral smear on day 7. Moreover, patients were asked to obtain simultaneously optional bone marrow aspiration after getting informed consents to show whether there will be any concordance with the steroid response and/or whether it can give any idea of the outcome.

Results: Steroid response rate on day 7 by peripheral smear was 91% (n=158) for the whole group. However simultaneously bone marrow MRD measurement was done in 22 of the 173 patients. There were 13 female and 9 male patient with a median initial WBC count of 8400/mm3 (1100-55300/mm 3), all were Calla+ pre B cell ALL (17 low risk ALL, 6 standard risk and 1 high risk ALL), all were in complete remission and all except one is alive at the time of the analysis. There were 10 patients receiving 10mg/kg/d HDMPX and 12 patients were in the group of 20mg/kg/d HDMPX. MRD levels were not statistically different on day 7 between these two groups. Furthermore all patients except 2(one in each group) were steroid responsive by means of peripheral absolute blast count <1000/mm³. Bone marrow MRD on day 15th and 42nd there were no statistically significant difference in each group(P=0.05). Although some of those patients in each group have high levels of MRD on day 7, interestingly they were all steroid responsive.

Summary/Conclusions: Our preliminary results suggest to think that MRD level on day 7 in a small group of low/standard ALL patients may not predict outcome.

E856 PONATINIB (PON) IN PHILADELPHIA CHROMOSOME (PH)-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): PRELIMINARY REPORT OF THE OPAL OBSERVATORY

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Background: Philadelphia positive acute lymphoblastic leukemia (Ph+ ALL) patients have improved since the introduction of tyrosine kinase inhibitors (TKI). Dasatinib was given in association with steroids at the dosage of 2mg/kg/d in both groups and continued with this dose till the end of 3rd week of induction phase. MRD levels were studied at the 15th, 22nd and 42nd days of induction according to the protocol. However, we also analysed steroid response rate by the peripheral smear on day 7. Moreover, patients were asked to obtain simultaneously optional bone marrow aspiration after getting informed consents to show whether there will be any concordance with the steroid response and/or whether it can give any idea of the outcome.

Methods: Dasatinib was given in association with steroids at the dosage of 140mg idie until the achievement of CR. FLAD regimen consisted of a three-days administration of Flu 30mg/m² followed by Ara-C 2000mg/m² and DNX 100mg/m². Dasatinib was administered again from the end of chemotherapy and G-CSF was given to all patients starting from day 4 until complete hematological recovery. FLAD was administered for up to two cycles. Minimal residual disease (MRD) was evaluated in all patients after each FLAD either by multicolor flow cytometry (MFC), RQ-PCR for VDJ rearrangements, and RQ-PCR for BCR/Abi.

Results: From January 2008 to December 2016, 8 Ph+ ALL at diagnosis (medi-an age 52 years) have been enrolled in this protocol. The median follow-up was 27 months. All patients received 70 days induction with Dasatinib + Steroids and achieved CR with complete hematological recovery. In all patients but one, however, BCR/Abi was still positive both on day 33 and on day 70. Three patients were MFC MRD positive on day 33 (one on day 70 also), whereas five patients achieved MFC MRD negativity on day 33. After the first FLAD course all patients achieved MFC MRD negativity, with four patients achieving also negativity for VDJ rearrangements and BCR/Abi transcript. FLAD was very well tolerated, with a median ANC and platelet recovery of 7.5 and 4 days, respectively. No patient experienced relapse so far and 5/8 patients proceeded to HSCT. Two patients are currently waiting for transplant. Overall, 6 patients are alive and in MRD negative CR at the time of analysis. One patient died at day +289 after SCT due to non-relapse mortality and one has died after the first FLAD in molecular CR because of an unrelated event.

Methods: Our preliminary results suggest to think that MRD level on day 7 in a small group of low/standard ALL patients may not predict outcome.
Background: PON, a third-generation tyrosine-kinase inhibitor (TKI), displays activity in de novo Ph-positive (Ph+) ALL and chronic myeloid leukemia (CML) in lymphoid blastic phase (LyBP), as shown when given as single agent in 42 patients (pts) with resistant disease in the PACE trial (Cortez, NEJM 2013), or combined to first-line chemotherapy in 58 pts (Jabbour, Lancet Oncol 2015; Sasaki, ASH 2016).

Aims: Because data are still limited to few selected pts, we analyzed the outcome of pts treated with PON in the real-life setting (OPAL observatory).

Methods: Pts were recruited if aged ≥18 years; with de novo Ph+ or CML-LyBP treated by PON alone or in combination for at least 1 treatment day, for relapsed or refractory disease, between Apr 2012 and Dec 2014 (Expanded Access Program). Twenty-one pts were analyzed (16 men and 5 women; 17 de novo ALL and 4 LyBP-CML), with a median age of 60 years (22-73). Time from first ALL or CML-LyBP diagnosis was 6 months (1-123). At PON initiation, 1 pt had primary refractory ALL, 15 pts were in first salvage (1 in second complete remission [CR] after chemotherapy, 3 in molecular relapse only), 2 in second salvage, and 3 in third salvage or beyond. Numbers of patients who had previously received 1, 2, 3, or 4 other TKIs were 4, 15, 1, and 1 respectively (14 pts had Ph+ ALL). We included pts with Ph+ ALL or Ph- CML-lymphoblastic leukemia (CML-LyBP) for at least 1 treatment day, regardless of response. E858

Results: Median duration of PON treatment was 3 months (5 days-30 months+). Out of the 19 pts who received PON for ≥4 weeks, 5 pts failed to reach CR, while 14 (78%) reached or maintained it. Molecular response was not reported uniformly. During induction by PON, 5 grade 3-4 events occurred in 4 pts (1 pulmonary infection; 1 acute renal failure; 1 pancreatitis; 1 hepatitis; 1 venous thrombo-embolic event; no arterial occlusive event). Post-induction therapy consisted in PON-based therapy in most pts. HSCT was performed in 5/pts. Overall survival rate in CR on PON, 1 pt died in CR from PON toxicity at 8 months and 11 pts ultimately experienced bone marrow relapse, all of them within 6 months after PON initiation, except 2 who relapsed at 13 and 27 months after HSCT. Two patients are alive in CR at 14 and 30 months, and 1 attained MRD negative.

Summary/Conclusions: Our series of resistant pts is comparable to the PACE study population by initial characteristics and high frequency of BCR-ABL mutations. CR was achieved in most pts, suggesting the role of PON as a bridge-to-transplant with a favorable risk-benefit ratio. Effective post-induction combination with agents such as cytarabine, rituximab, and anti-CD20 antibodies may improve outcome. Further knowledge gained from multiple cytokine and adhesion molecule evaluation could help to improve treatment outcomes.

Aims: The aim of this study was to evaluate baseline levels of cytokines, cytokine receptors and adhesion molecules in newly diagnosed acute lymphoblastic leukemia (ALL) patients and to assess their correlation with baseline characteristics and prognostic factors.

Methods: A total of 30 newly diagnosed ALL patients (median age 46, range 22–75 years, 20 males) were included. We evaluated serum levels of 31 analytes, specifically 21 cytokines, 4 soluble cytokine receptors, 5 soluble adhesion molecules and Matrix Metalloproteinase-9. From cytokines, we measured Interleukins (IL-1α, IL-1β), IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-23), Epidermal Growth Factor (EGF), Granulocyte Macrophage Colony Stimulating Factor (GM-CSF), Interferon-γ (IFN-γ), Macrophage Inflammatory Protein-1α (MIP-1α), Monocyte Chemotactic Protein-1 (MCP-1), Tumour Necrosis Factor (TNF-α), Vascular Endothelial Growth Factor (VEGF) and soluble receptors for IL-2 (sIL-2Ra), IL-6 (sIL-6R), TNF-α type I and II (sTNFR-1, sTNFR-2). From soluble adhesion molecules, we measured E-Selectin (E-SEL), L-Selectin (L-SEL), P-Selectin (P-SEL), Intercellular Adhesion Molecule-1 (ICAM-1) and Vascular Cell Adhesion Molecule-1 (VCAM-1). All analytes were measured by biochip array technology on Evidence investigator analyzer (Randox). Serum levels of tested analytes were correlated with baseline characteristics such as age, sex, risk group according to GAML (SR 9, HR 9, VHR 12 patients), full blood count parameters (including percentage of blasts), biochemical parameters (LDH, CRP), response to induction therapy (CR rate after induction), progression-free survival (PFS) and overall survival (OS). Statistical evaluation was done by a professional statistician using software R 3.3.2 (R Core Team 2016).

Results: Comparing analytes with baseline characteristics, we found significant negative correlations between IL-7 and leukocyte count (r=-0.633; p=0.032), percentage of blasts in peripheral blood (r=-0.695; p=0.004) and LDH (r=-0.604; p=0.075). Furthermore, we found significant positive correlations between IL-7 and leukocyte count (r=0.601; p=0.0001), MCAM-1 and LDH (r=0.864; p=0.012). Correlations with baseline risk stratification according to GAML did not reach statistical significance. In the study population, CR rate after induction was 86% (MRD negative in 29%), 1-year PFS 68% and 1-year OS 73% (2 patients died during induction therapy). Higher levels of EGF were associated with failure to achieve CR after induction therapy (r=0.689; p=0.073). So far, no significant correlations between baseline analyte levels and inferior PFS or OS were found. In newly diagnosed ALL patients, we found statistically significant correlations between sTNFR-1 and sTNFR-2 (r=0.805; p=0.0001), IL-1α and IL-4 (r=0.700; p=0.008), TNF-1 and sTNFR-2 (r=0.657; p=0.037), sTNFR-2 and VCAM-1 (r=0.652; p=0.044). Correlations between cytokines and clinical outcome did not reach significant levels.

Summary/Conclusions: Our findings show that serum levels of IL-7 and VCAM-1 are associated with some baseline characteristics of newly diagnosed ALL patients and EGF with response to induction therapy. Better understanding of leukemia microenvironment is essential for development of new treatment approaches. Further studies in this field are warranted.

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IMATINIB VS. DASATINIB FOR OUTCOMES AFTER ALLOGENEIC STEM CELL TRANSPLANTATION FOR PATIENTS WITH PH+ ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: The survival of the patients with Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ALL) who received allogeneic stem cell transplant (allo-HSCT) has improved over the development of tyrosine kinase inhibitors (TKIs). Currently, Imatinib (IMA) and Dasatinib (DAS) are widely used for the treatment for Ph+ALL. However, there has been no data comparing the outcomes between the patients who received allo-HSCT and the two distinctive TKIs respectively.

Aims: We conducted a retrospective analysis for comparing the two TKIs for the outcome after allo-HSCT.

Methods: Clinical data of patients were retrospectively collected from Hokkaido University Hospital and Sapporo Hokusy Hospital. The patients' eligibility was as follows: diagnosed as Ph+ALL, aged more than 16 years, and received allo-HSCT between 1990 and 2016 and first time for SCT.

Results: Sixty-six patients were eligible for the study. Fifty-six out of the 66 were administered TKIs (TKI group) and the remaining ten who developed Ph+ALL in the event in the patients were treated without TKIs (non-TKI group). Overall survival rates were not different between the two groups. Of the 56 patients in the TKI group, 39 received IMA (IMA-pts), and the remaining 17 received DAS (DAS-pts). Compared with DAS-pts, IMA-pts received allo-HSCT in relatively older years of age, more frequent myeloablative conditioning regimen, and cyclosporine-containing, not tacrolimus-, regimen for GVHD prophylaxis more frequently. Overall survival rates, such as age, disease status at HSCT, or stem cell source were not significantly different between the two groups. Incidences of Neutrophil engraftment and acute GVHD incidence were not statistically different between IMA-pts and DAS-pts. Incidence of chronic GVHD was marginally increased in IMA-pts (IMA; 63%, DAS; 33%; P=0.08). At the median follow-up of 531 days, survival was not different between the two groups by univariate analysis (Logrank, P=0.16). However, by multivariate analysis using Cox regression model for adjusting confounding factors, including, overall survival was superior for IMA-pts (Hazrad ratio: 0.32 (0.11-0.94), P=0.04). Incidences of transplant-related mortality and relapse were not different between the groups, even though relapse rate tended to be increased in DAS-pts (IMA: 16.1%, DAS: 47.1%, Gray P=0.2).

Summary/Conclusions: Our analysis suggests that overall survival may be superior for the Ph+ALL patients treated with allo-SCT and IMA compared with those with DAS. There are some limitations for our analysis due to retrospective fashion and relatively small number of the patients analyzed. Therefore, prospective study comparing survival of the Ph+ALL patients treated with the two distinctive TKIs before HSCT is needed.

E860

IS OLDER AGE AN EXCLUSION CRITERION FOR ALLOGENEIC HEMATOPOIETIC STEM-CELL TRANSPLANTATION IN PATIENTS WITH PHILADELPHIA-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA?

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Background: Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL) is diagnosed more often in older than in younger patients. This type of the acute lymphoblastic leukemia is characterized by very aggressive course of the disease. All clinical recommendations for such conditions indicate allogeneic bone marrow transplantation (allo-BMT) as a treatment of choice. After achieving complete remission. The addition of tyrosine kinase inhibitors (TKI) to chemotherapy has dramatically improved the long-term outcome in Ph+ acute lymphoblastic leukemia patients. Nevertheless whether to administer chemotherapy at all and if yes – how intensive it should be, is still the matter of debate. We have conducted two consecutive trials in Ph+ ALL aiming to evaluate the efficacy of more and less intensive chemotherapy approaches in combination with constant non-stop 600 mg Ima-tinib. All patients in both protocols with suitable donors underwent hemopoietic stem-cell transplantation (HSCT).

Aims: To analyze the effectiveness of RALL–2009+TKI and RALL–2012+TKI protocols in Ph+ ALL patients with or without HSCT. To analyze the efficacy of treatment with or without transplantation regarding the patient’s age.

Methods: From 2010 January to 2017 January, 35 new Ph+ ALL cases were diagnosed in 3 centers of the RALL–group. From 2010 to 2012, 12 Ph+ ALL patients were treated according to RALL–2009 protocol (ClinicalTrials.gov: NCT01193933) with concurrent administration of Imatinib. This protocol includes 8 cytostatic drugs and no intervals between treatment phases. Since 2012 till now 23 pts were included in ongoing RALL–2012 protocol, based mainly on 600 mg Imatinib with prednisolone, VNCR, L-asp, followed by 6-MP and MTX. Both protocols suggested the shift to Dasatinib (100-140mg) after non-achievement of MolCR on day 70 of treatment. MolCR was stated if bcr/abl chimeric transcript was <0.01% by PCR with 10-4 sensitivity. All patients were considered as candidates for allogeneic HSCT if HLA-identical donor was available. 13 pts (37%) underwent allo-HSCT as the first-line therapy. 1 autologous, 5 matched related and 7 matched unrelated.

Figure 1.

Results: MolCR on day 70 was achieved in 36% and 59% in RALL–2009 (n=4) and RALL–2012 (n=13) respectively. Death on therapy (within 2 months of induction/consolidation) was registered in 2 cases on less intensive RALL-2012 protocol and 2 cases on RALL–2009. Hematological CR was achieved in 30 (85.7%) of 35 pts (except four early deaths and 2 refractory cases). There was one allogeneic HSCT in MolCR on the first protocol. Allo–HSCT was carried out in 5 of RALL–2009 protocol pts and in 9 of RALL–2012. The major issue was the non-relapsed mortality after unrelated allo–HSCT in 3 older pts (49, 56 and 59 years old) who were included in RALL–2012 (aGVHD and severe infections, at a median +4 months after HSCT and more than 12 months of CR duration). The 5y overall survival (OS) and relapse-free survival (RFS) for all pts constitutated 54,6% and 40,4% respectively. The long-term outcome on both protocols (RALL–2009 and RALL–2012) was similar: OS – 62.8% vs 49,4% (p=0,6), RFS – 55,7% vs 45% (p=0,7), respectively. In order to evaluate the impact of allogeneic HSCT we performed a comparison of transplanted and non-transplanted patients by a landmark analysis. The landmark was chosen as the median time from CR to allo–HSCT – 4,3 mo (3-16 mo). So, the 5y OS from landmark was 53,3% for transplanted patients and from day of HSCT – 65,6% in transplanted (p=0,18), and RFS was 25% vs 62,5 (p=0,19), respectively. OS for older pts (>45 y) was 40% vs 25% in transplanted vs non-transplanted group of the pts, and RFS was 25% vs 66,6%, respectively. OS in younger (45-65 y) pts was 83,3% vs 58,9% for transplanted vs non-transplanted pts, EFS was 77,1 vs 21,4%, respectively.

Summary/Conclusions: The results very pessimistic in older (>45 years) patients who received HSCT. The contrary was observed in younger adult patients with very good results after HSCT – OS was 83,3% and EFS 77,1%. We conclude that patients aged>45y should continue chemotherapy without allogeneic HSCT or may be we could apply autologous HSCT for that group of the patients.

E861

TARGETABLE BLINATUMOMAB + TYROSINE KINASE INHIBITORS TREATMENT IN RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS: CLINICAL EFFECTIVENESS AND PERIPHERAL LYMPHOCYTES SUBPOPULATIONS KINETICS

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Background: Blinatumomab is a bispecific monoclonal anti-CD3/CD19 antibody which has clinical activity in relapsed/refractory Ph-positive acute lymphoblastic leukemia (ALL) as monotherapy. Combination of Blinatumomab with
tyrosine kinase inhibitors (TKI) is the promising approach in treating Ph-positive ALL. Some other rearrangements like **IKZF1** in Ph-like ALL, **FLT3** and **JAK2** in Ph-negative ALL are the potential targets to some TKIs.

**Aims:** To demonstrate effectiveness and toxicity profile of Blinatumomab+TKI treatment. To evaluate peripheral blood lymphocytes subpopulations kinetics during blinatumomab treatment.

**Methods:** From October 2015 to February 2017 10 patients (pts) aged from 24 to 42 (median 31), 7 female and 3 male, with relapsed/refractory ALL were treated in our center. The diagnosis was relapsed ALL in 8 pts (7 – overt hematological, 1 – cytogenetic relapse) and persistent/increasing minimal residual disease of ALL in 2 pts. All pts had strong CD19 positivity. 8 pts was diagnosed as Ph-positive ALL (p190), 1 – Ph-like ALL (IKZF1 rearranged), 1- FLT3+ ALL. Two pts has T315I ABL mutation. In all pts blinatumomab continuous infusion + TKI therapy was started. Blinatumomab dose during 1st week of 1st cycle was 9 mcg/day, 28 mcg – subsequent three weeks. Blinatumomab dose in subsequent 4-weeks cycles was 28 mcg/day. 7 pts were treated with TKI Dasatinib, 1 – Bosutinib (Dasatinib/Nilotinib intolerant), 1 – Ponatinib (T315I), 1 – Sorafenib (FLT3+). ATRA was added to Dasatinib in 1 pt with IKZF1 rearranged Ph-like ALL. 1 pt received 1 cycle of 4 weeks blinatumomab, 1 pt – 2 cycles, 6 pts - 4 cycles, 2 pts – 5 cycles. TKIs were administered continuously in all pts. T-helper, T cytotoxic, T-regulatory and NK cells were measured by flow cytometry in every week during all cycles of blinatumomab treatment.

**Results:** No one pt has neurological toxicity of any grade. All pts has significant decrease of normal IgG level and all of them received intravenous human normal immunoglobulin replacement. Palmoplantar syndrome in one pt on sorafenib completely resolved after temporarily TKI discontinuation. Disarrhythmia in 1 pt on dasatinib/nilotinib completely resolved on bosutinib. 8 pts achieved molecular remission (MoCR), one pt – cytogenetic remission and one pt with T315I progressed to overt hematological relapse. T-helper and T-lymphocyte subpopulations were on or below of lower limit of normal range. T- cytotoxic and NK subpopulations gradually returned into normal range (Fig. 1). AlloBMT was performed in 4 pts. Three pts are awaiting alloBMT and three are continuing Blinatumomab + TKI treatment.

**Summary/Conclusions:** Lowering toxicity in non-chemotherapy treatment has its significance in such a heavily pretreated patients with relapsed ALL. The treatment has high MoCR rate and low toxicity profile. Treatment effectiveness correlated with T-helper and T-regulatory subpopulations exhaust. T-cytotoxic and NK cells subpopulations restoring also correlated with clinical effectiveness.

E863

**NOVEL CRLF2 MUTATIONS AND CLINICAL SIGNIFICANCE IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA**

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**Background:** Cytokine receptor-like factor 2 (CRLF2) plays an important role in the development of normal B lymphocytes, which can mediate early B cell proliferation and survival. CRLF2 overexpression and rearrangement have been observed in acute lymphoblastic leukemia (ALL), and they are reported to contribute to oncogenesis and unfavorable outcome in ALL. We reported that CRLF2 overexpression in the patients without CRLF2 rearrangement, indicating the reason other than CRLF2 rearrangement is responsible to the CRLF2 overexpression. There is few reported CRLF2 mutations in adult ALL.

**Aims:** To investigate the mutations of CRLF2 and its clinical significance in adult ALL without CRLF2 rearrangement.

**Methods:** The 129 patients’ BM samples (95 B-ALL, 33 T-ALL and 17 B-T-ALL) were collected between April 2010 and Jan 2015 at the First Affiliated Hospital of Nanjing Medical University. The ALL diagnosis was made according to the FAB criteria. The immunophenotypic and molecular characteristics were analyzed using a 12-color immunophenotyping assay and molecular FISH analysis. The frequency, positions, types and clinical significance of CRLF2 mutations were analyzed. For qualitative parameters, overall group differences were analyzed using a χ2 test. All statistical analyses were performed using SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA). P<0.05 was considered to indicate statistical significance.

**Results:** Six novel CRLF2 mutants were detected in the 129 patients without CRLF2 rearrangement, which were L86K (0.8%), R186S (7.8%), P224L (8.5%), W225C (0.8%), and two silent mutations F232F (0.8%) and A112 (12.4%). The overall rate of CRLF2 mutation was 26.6%. Exon1, exons 5 and exons 6 were analyzed in B- and/or T-ALL patients; but no mutations were detected in exon2 and exon4. None of these mutations were reported in the COSMIC and SNP databases. The patients with R186S, P224L mutations showed significant differences with that of non-mutant patients in sex, age, white blood cell count, hemoglobin level, and platelet count. The median neutrophil count in the patients with P224L mutation

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haematologica | 2017; 102(s2) | 355
was lower than that of non-mutation (8.53×10^9/L vs 28.9×10^9/L, P=0.032). The positive rate of Ph chromosome in patients with R186S was lower than that without the mutant (10.0% vs 31.8%, P=0.018). In addition, the incidence of splenomegaly in patients with R186S and P224 L mutants was lower than that in non-mutant patients (0.0% vs 29.5%, P=0.026; 0.0% vs 29.7%, P=0.034, respectively). The B-ALL patients with L86I mutant had myeloid antigen expression, high white blood cell count (248.4×10^9/L) and low platelet count (10×10^9/L), and relapsed in two months after the first induction chemotherapy; and the overall survival was only 2 months. The patient with W255C mutation did not achieve complete remission (CR) with the first induction chemotherapy.

Our data indicated that the genetic mutations were identified in adult ALL patients and may associate with clinical outcome, such as mutation relapsed in 6 months. Interestingly, the patient with silent mutation, A11A showed higher age (46 years), without the mutant (10.0% vs 75.3%, P=0.035). CD22 (93.3% vs 47.4%, P=0.020) were lower than those without the mutation; and the patient with F232F mutation relapsed in 6 months.

**Summary/Conclusions:** Six novel CRLF2 genetic mutations were identified in adult ALL patients and may associate with clinical outcome, such as CRLF2 R186S indicating favorable, while L86I and W255C indicating poor outcome. Our data indicated that the CRLF2 mutations may be new prognostic markers and play an important role on oncogenesis in ALL.
Background: Mixed Lineage Leukemia’s (MLL’s) are characterised cytogenetically by reciprocal translocations of the MLL gene and clinically by unfavourable outcomes. Evidence indicating that MLL leukemia’s are resistant to apoptosis encourages the identification of novel drug targets. 

Aims: Using cord blood (CB) CD34+ cells (control) and CB CD34+ cells expressing MLL-AF9, we sought to determine the potential role of BTK in the development and progression of MLL+ leukemia. Further aimed to uncover possible downstream target/s of BTK, improving the therapeutic efficacy of the drugs used. 

Methods: Experiments were performed using control and MA9.3 cells and leukemic blasts from 3 AML (MLL+) patients. Signalling events were evaluated by immunoblotting. p65 mediated BTK expression was determined by promoter assays. Cells were treated with specific inhibitors of BTK (Ibrutinib (IBR): 0.25, 0.5, 1.0 and 2µM) in combination with Daunorubicin (DAU 5nM) or RAC (NSC 23766 (NSC): 5, 10, 15 and 20µM) for 48 hrs and cell viability was assessed using Annexin V/ Sytox-Blue based flow cytometric analysis.

Results: To determine the relevance of BTK as a therapeutic target in MLL+AML, we examined the whole cell lysates (WCL) from control cells, two clones of CB expressing MA9 (MA9.3 and .6) and leukemic blasts from the 3 AML patients. Activated BTK (pY223) was detectable in both the clones of MA9 and MLL+AML samples. Interestingly, the cells demonstrated activation of p65 (pS536) but not in control cells. To address if activated p65 could potentially drive BTK expression, we performed BTK promoter assays with reporter construct and empty vector. MA9.3 cells electroporated with test construct demonstrated significantly higher transcriptional activity. At the protein level, p65 inhibitor treatment (MG132 or Bay 11-7082) reduced total BTK expression, indicating that the activated p65 translates to the expression of BTK. Treatment of control and MA9.3 cells with various concentrations of IBR for 48 hrs induced a dose-dependent reduction of cell viability (Annexin V and Sytox blue negative). We further sought to determine if the use of IBR in combination with Daunorubicin would further sensitize MA9.3 cells. The apoptotic rate of the cells with combination treatment was significantly higher than that of cells treated with IBR or DAU alone. The coefficient of drug interaction (CDI) values indicated that IBR-DAU combination synergistically reduced cell viability (CDI > 1.0-antagonistic; < 1.0: synergistic and = 1 additive effect). Recent studies suggested RAC-GTPase signaling may also represent a target in AML, particularly in the context of FLT3-ITD driven AML (Wei J et al., Cancer Cell 2008 and B Mizukawa et al., Blood 2011). This intrigued us to test if BTK is possibly upstream of RAC. We measured activation of the GTPase RAC via active RAC pull down assay. Interestingly, treatment with IBRUs significantly reduced RAC activation, positioning BTK upstream of RAC. In line with observations reported earlier, we observed that MA9.3 cells are responsive to RAC inhibitor, NSC. This effect of NSC on cell viability was further potentiated in combination with IBR (0.5µM). CDI values once again indicated that the drugs together have a synergistic effect on reducing the cell viability.

Summary/Conclusions: Taken together, our data support a biological link between NFkB, BTK and RAC pathways in the modulation of cell survival in MLL-rearranged AML cells. Aberrantly active p65 drives the expression of BTK and contributes to the progression of the AML. Combination treatment using IBR-DAU and IBR-NSC might be a promising therapeutic strategy, minimizing high drug dose-related side effects but increasing the therapeutic efficacy.

**ES86**

A PRECISION MEDICINE PLATFORM FOR ACUTE MYELOID LEUKEMIA TO HELP UNRAVELLING THE MOLECULAR ADDICTIONS OF FLT3-ITD-DRIVEN AML

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Background: Acute myeloid leukemia (AML) is an aggressive disease with poor prognosis (Tzelepis et al. 2016). No single driver mutation is present in all cases of AML, making its treatment a challenge (The Cancer Genome Atlas Research Network 2013). The knowledge of standard of care for AML is an argued one, furthered to uncover ic-based treatment that has remained unchanged for the past 30 years (Longo et al. 2015). Weak or elderly patients might not be eligible for intensive treatment, leading to poor survival rates. Many such patients are labeled as ‘untreatable’, although a portion of them could benefit from specific, individualized treatment. A personalized medicine strategy can help to find the specific treatment for these ‘untreatable’ AML patients.

Aims: Drug-driven personalized medicine aims to directly test the sensitivity of primary cancer cells taken from individual AML patients to a selection of targeted cancer drugs, compare these results with drug sensitivities of healthy donor samples and select the most effective drug for each patient. This approach considers any combination of mutations or epigenetic changes that might not be found in the standard sequencing panels, an advantage when dealing with such a heterogeneous disease. Proof of principle of this strategy was recently demonstrated by FiMM (Helsinki, Finland) (Pernovska et al. 2013), not only providing immediate clinical benefit to leukemia patients, but also identifying drugs that can potentially be repurposed for future treatment of patients.

Methods: We have established a drug-driven personalized medicine platform for AML where we check the ex-vivo drug sensitivity and resistance of bone marrow primary cells to a panel of around 400 drugs and drug combinations covering the standard of care, as well as many clinically available and emerging molecularly targeted compounds. We calculate the IC50 values for all the drugs for each individual donor or patient, and then the differential drug sensitivity scores, selecting the drugs that affect preferentially the cancer cells when compared with healthy cells. To date we have successfully processed 6 healthy donors and 66 AML samples identifying subgroups of patients who respond with a similar dynamic to certain classes of drugs, as the subgroup of cells carrying internal tandem duplications in the receptor tyrosine kinase FLT3 (FLT3-ITD).

Results: FLT3 activating mutations, particularly FLT3-ITD, have been observed covering the standard of care treatments, cancer chemotherapeutics as well as inhibitors such as Ganetespib compared to healthy donors and any other subgroups of leukemia. In addition, HSP90 inhibitors specifically sensitize FLT3-ITD-expressing bone marrow-derived cells to TKIs, whereas cells derived from healthy donors are unaffected. HSP90 inhibitors also preferentially eradicate a population of patient-derived FLT3-ITD+ AML cells expressing leukemia stem cell markers.

Summary/Conclusions: In summary, our study reveals a molecular basis for HSP90 addiction of FLT3-ITD-driven AML and provides a rationale for treatment of this form of AML with HSP90 inhibitors.
E868

CLONAL EVOLUTION OF FLT3-ITD POSITIVE AML AT DIAGNOSIS AND RELAPSE IN PATIENTS TREATED WITHIN THE CALGB10603 (RATIFY) AND AMLSG 16-10 TRIALS

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Background: Internal tandem duplications (ITD) in the receptor tyrosine kinase FLT3 occur in about 22% of patients (pts) with acute myeloid leukemia (AML) and confer a poor prognosis depending on the mutational load. The multi-targeted TKI midostaurin has been shown to improve outcome in FLT3-ITD positive (FLT3-ITD++) and FLT3-ITD- mutated (FLT3-TKD300++) pts in combination with intensive chemotherapy [CALGB 10603 (RATIFY) trial], thus representing a promising targeted treatment approach. However, a significant number of pts relapse after initial response due to yet unknown mechanisms.

Aims: To study the clonal evolution in FLT3-ITD++ pts treated in the AMLSG16-10 (NCT01477660) or CALGB 10603 (RATIFY, NCT00651261) trial in paired samples obtained at diagnosis (Dx), complete remission (CR) and relapse (Rel) by whole exome sequencing (WES).

Methods: WES was performed in 17 FLT3-ITD++ pts using the Nextera Rapid Capture Exome kit (illuminla) for library preparation followed by sequencing on a Illumina HiSeq 2000. 6 pts were treated in the RATIFY trial receiving either midostaurin (TKDmut) or placebo combined with intensive chemotherapy during induction and consolidation; 11 pts were treated in the AMLSG16-10 trial, which includes midostaurin or placebo combined with intensive chemotherapy during induction and consolidation followed by a one-year maintenance therapy with midostaurin; 4 pts in the AMLSG16-10 trial received alemtuzumab hegatopoietic cell transplantation. The presence of FLT3 and NPM1 mutations (mut) and the allelic ratio (AR) of FLT3-ITD were analyzed according to standard protocols.

Results: The median AR of FLT3-ITD was 0.51 (0.10-18.94) and 0.54 (0.07-26.31) at Dx and Rel, respectively. Loss of FLT3-ITD was observed in 5 pts; changes of the ITD clone at Rel occurred in 7 pts. Of those, 5 pts had a change of both FLT3-ITD mut and 1 pt gained an additional ITD clone at Rel. 3 pts had a D835V FLT3-TKD300 that was lost at Rel. 6 pts had a NPM1mut that persisted at Rel in all pts. Using WES, 301 mut (226 missense, 24 nonsense, 41 indels, 6 splice sites, 4 unique) were identified. The average coverage was 125 (186-67) among all samples. 131 (43%) mut were present at both time points (Dx/Rel). 60 mut were found only at Rel. 73 (24%) mut were detected only at Rel and 14 mut with only 1 read at Dx. Besides FLT3-ITD, the average number of mut per sample (Dx or Rel) was 13. Mut were most frequently observed in genes related to signaling (23%), transcription (20%), DNA methylation (9%), chromatin remodeling (9%), components of the meiotic recombination (4%), Pre-leukemias (DNMT3a, TET2, IDH1/2) were detectable in 10 pts at both time points and persisted at CR in 7 pts. Recurrent mut in transcription change genes occurred in 8 pts at Dx and Rel, with W71 mut being most frequent (n=7). Mut in signaling related genes present at both time points included NRAS (G12V/D) and NFT1 mut. At the time of Rel, gene mut frequently referred to signaling (34%) including a KRAS (G13D) and a KIT (D816V) mut, both in pts with loss of FLT3-ITD at Rel.

Summary/Conclusions: Analyzing the clonal evolution of FLT3-ITD++ AML, known pre-leukemic mut were stably detectable at Dx and Rel in most pts, whereas novel acquired gene mut were observed in 35% of the pts. We have investigated more comprehensively pathways underlying therapy resistance with a focus on TKI treatment, larger cohorts of pts are currently analyzed to determine the detection of recurrent mutational patterns.

E869

MICROENVIRONMENT SECRETED PROTEINS MEDiate RESISTANCE TO TARGETED THERAPY IN PRIMARY AML CELLS

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Background: The bone marrow stromal microenvironment (BMSM) plays an important role in the pathophysiology of acute myeloid leukemia (AML). This is demonstrated by primary AML blasts dependence on stromal conditioned media to survive long-term culture. Although some of the components of the stromal secreteme (the totality of secreted proteins by biological cells) that augment AML survival are known, the precise molecular mechanisms of the stromal-blast interactions are not fully defined.

Aims: i) identify proteins secreted by bone marrow stromal cells that mediate AML survival (TKDmut) ii) Investigate global changes in signalling pathway activity induced by stromal factors in primary AML; iii) Validate the functional significance of these interactions through targeted inhibition of BMSM activated signalling pathways.

Methods: We used primary AML cells and established cell lines. Four different human AML cells were grown individually or in co-culture with a mouse bone marrow stromal line (MS-5). The resulting conditioned medium from these experiments (4 AML lines alone, 4 AML lines + MS-5, MS-5 alone) was purified to obtain the secreteme (in triplicate). Proteins in these secretemes were quantified using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). Protein sequence searches against mouse and human proteomes allowed for discrimination between the mouse stromal and human AML proteins. Guava EasyCyte Flow Cytometry was used to measure the viability and proliferation of these cell populations, assessing the capabilities of the identified factors above on primary AML cells (n=6) as well as the effects of kinase inhibitors (sunitinib, trametinib, midostaurin) on cell proliferation and apoptosis.

Results: Initially by comparing secretemes of the four AML lines (on their own or in MS-5 co-culture) we identified 520 bone marrow stromal proteins and 293 AML blast proteins. From these, six stromal proteins were selected (including two secreted proteins) and we were able to dissect specificity for discrimination between the mouse stromal and human AML proteins. Guava EasyCyte Flow Cytometry was used to measure the viability and proliferation of these cell populations, assessing the capabilities of the identified factors above on primary AML cells (n=6) as well as the effects of kinase inhibitors.

Summary/Conclusions: This proteomic approach has allowed identification of a panel of key proteins (including S100-A11, CTGF, BMP-1) secreted by the stromal cells that modulate cell signalling and cell fate in AML blasts. Using a proteomic approach to study global cellular effects we were able to dissect specificity for discrimination between the mouse stromal and human AML cells. These six proteins were used in varying combinations to determine their effect on growth of primary AML cells from patients (n=6). We also analysed the phosphoproteomes of primary AML cells displaying the maximal effects on growth in response to the six factors above to determine the underlying biological mechanisms. These studies have shown that several different pathways are activated as a result of secreteme treatment including mTOR and MAPK. Primary AML cells were sensitive to targeted inhibition of these pathways. However, the inclusion of stromal secreted factors to the same AML cells would induce sensitivity to another kinase inhibitor and insensitivity towards the previously effective inhibitor.

E870

CHARACTERIZATION OF FLT3 MUTATIONS AT DIAGNOSIS, REFRACTORY DISEASE OR RELAPSE IN AML PATIENTS TREATED WITH MIDOSTAURIN WITHIN THE CALGB 10603 (RATIFY) AND AMLSG 16-10 TRIALS


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Background: FLT3 mutations (mut) and the FLT3-ITD at Rel.

Aims: i) Identify genes present at both time points included NPM1 (G13D) and a KIT (D816V) mut, both in pts with loss of FLT3-ITD at Rel.

Summary/Conclusions: Analyzing the clonal evolution of FLT3-ITD++ AML, known pre-leukemic mut were stably detectable at Dx and Rel in most pts, whereas novel acquired gene mut were observed in 35% of the pts. We have investigated more comprehensively pathways underlying therapy resistance with a focus on TKI treatment, larger cohorts of pts are currently analyzed to determine the detection of recurrent mutational patterns.
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Background: Internal tandem duplications (ITD) and mutations (mut) in the tyrosine kinase domain (TKD) of the receptor tyrosine kinase FLT3 occur in about 25% of acute myeloid leukemia (AML) patients (pts). FLT3-ITD is associated with an unfavorable prognosis in particular in pts with a high allelic mutant to wildtype ratio (AR>0.5) as well as localization of the ITD in the beta1-sheet of the receptor. FLT3 is targetable by tyrosine kinase inhibitors (TKI) and the combination of chemotherapy with the TKI midostaurin has been recently investigated within the CALGB 10603 RATIFY trial and is still under investigation within the AMLSG 16-10 trial. Aims: To study the FLT3mut status at the time of diagnosis (Dx), refractory disease (RD) and relapse (Rel) in AML pts treated within the CALGB 10603 RATIFY, NCT00651261 and AMLSG 16-10 (NCT01477606) trial with regard to AR of FLT3-ITD and FLT3-TKDMut, loss of FLT3-ITD and FLT3-TKDMut and change of ITD clones (ITD insertion site, length, number of clones).

Methods: FLT3-ITD and FLT3-TKDMut were detected using Genescan-based fragment-length analysis according to standard protocols. In the randomized phase-III RATIFY study, FLT3mut pts were treated with induction (daunorubicin/cytarabine) and consolidation (high-dose cytarabine) and maintenance therapy in FLT3-ITD positive pts.

Results: In total, 83 pts were analyzed, of which 33 were treated in the RATIFY and 50 within the AMLSG 16-10 trial. 36 pts had RD and 47 pts had relapsed. FLT3-ITDwas present at diagnosis in all pts treated in the AMLSG 16-10 trial; one pt had an additional FLT3-TKDMut. Pts entered in the RATIFY trial had either a FLT3-ITD (n=22), a FLT3-TKDMut (n=9), or both (n=2). The median AR of FLT3-TKDMut at Dx was 0.82 (0.70-2.66) and the majority of pts showed loss of FLT3-TKDMut at RD or Rel (n=9/12; 75%). In relapsed pts, loss of FLT3-ITD occurred in 14 (34%) pts. There was no significant difference between the median FLT3-ITD AR at Dx [0.82 (0.10-18.94)] and Rel [0.65 (0.07-38.75); p=0.98]. A subset of the ITD clones was found in 14 (36%) pts at Rel, with switch of the ITD insertion site or length in 8 (21%) pts. 8/14 pts with change of the ITD clone at Rel had multiple ITD clones at Dx. For 35 FLT3-ITDPositive pts with refractory AML, FLT3-ITD loss was observed in 17 (49%) pts. The median AR of FLT3-ITD was significantly lower at the time of RD [0.29 (0.05-2.37)] compared to Dx [0.82 (0.05-9.81); p=0.02]. The ITD clone changed in 5 (14%) pts with RD. In pts with shift of the ITD clone at RD (n=5) or Rel (n=14), no significant difference of the median ITD length was observed (p=0.84).

Summary/Conclusions: Comparing the FLT3-ITD status at Dx, at the time of RD or Rel, we found a lower median AR of FLT3-ITD in pts at RD compared to Dx, whereas no significant change of AR was observed at Rel. In addition, loss of FLT3-ITD was observed in 49% of pts at RD and in 36% of pts at Rel. These findings suggest that the FLT3-ITD clone can be targeted in a significant number of pts and other clones might mediate resistance to treatment. We also observed a switch of the ITD clone in about 20% of pts with RD, indicating the presence of ITD clones which are resistant to conventional chemotherapy treatment. Despite the small number of TKD mutations in our study, it was remarkable that most of the TKDs (75%) were lost at the time of RD or Rel.

E871

A NOVEL PML-RARF FUSION IN ACUTE PROMYELOCYTIC LEUKEMIA

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Background: Acute promyeoletic leukemia (APL) is a subtype of acute myeloid leukemia (AML) characterized by specific translocation involving retinoic acid receptor alpha (RARα) locus. Retinoic acid receptor (RAR) is a member of nuclear receptor family, and has three types of isoforms such as RARA, retinoic acid receptor beta (RARβ) and retinoic acid receptor gamma (RARγ). The cerebrospinal fluid derived blasts (90%) of APL have clonal hypermethylation of the oncogenic properties of the artificial PML-RARF fusion gene was observed in an in vitro study, there has been no report on the PML-RARF fusion in human APL patients.

Aims: We report here a novel PML-RARF rearrangement in a patient with AML displaying a similar morphologic and immunophenotypic features of the classic hypergranular APL.

Methods: Whole genome sequencing (WGS) and further analysis of mRNA and gDNA were performed to clarify the atypical gene rearrangement observed by karyotyping and FISH.

Results: Laboratory and immunophenotypic analysis results suggested the classic APL with hypergranular type. A clonal translocation t(12;15)(q13;q22) was identified by karyotyping. No evidence of fusion of PML-RARA was detected by RT-PCR and PML-split was found on FISH analysis using PML-RARA dual color dual fusion probes. WGS analysis performed to clarifying the partner gene of PML located on chromosome 12q13 strongly suggested a PML-RARF fusion. RT-PCR following sanger sequencing were performed to verify the presence of PML-RARF fusion transcript, then two kind of transcripts was detected, one with the fusion of PML exon 3 and the middle part of exon 1 of RARG and the other with the fusion of PML exon 3 and exon 2 of RARG. The breakpoint of the DNA was clarified on intron 3 of PML and 5' region of RARG. Despite of ATRA treatment for 9 days, cell count did not show any response. Then induction chemotherapy composed of idarubicin and cytarabine was combined on ATRA. ATRA was finally stopped after 18 days, then cytogenetic remission was acquired day 36 after induction therapy.

Summary/Conclusions: We first report the presence of PML-RARF fusion in a human APL patient. This report supports the possibility of a new molecular mechanism involving RARG not RARA in APL and suggests the need of different therapeutic approach for this variant case showing the potential ATRA resistance.
Bone marrow microenvironment (BMM). Survival of patients with AML is presently poor; two-thirds of younger adults and 90% of older adults die of their disease. Even in patients who achieve remission with chemotherapy, relapse is common and occurs from minimal residual disease sequestered in protective niches in the BMM. Reciprocal interactions between that of the AML and bone marrow mesenchymal stromal cells (BM-MSC) are central to the survival and propagation of blasts through the bone marrow microenvironment. Promotion of quiescence in malignant cells as well as the activation of anti-apoptotic and pro-survival pathways.

**Aims:** To investigate how BM-MSC are programmed by AML to generate a pro-tumoral environment.

**Methods:** Primary AML and BM-MSC were isolated from the pelvis of AML patients following informed consent and under approval from the UK National Research Ethics Service (LRCEref07/H0310/146). Low input RNASeq of 10 AML BM-MSC and 10 healthy BM-MSC (taken from the pelvis of patients undergoing elective hip replacement surgery) was performed following CD271 Magnetic Cell Separation solution instructions. In-vitro co-cultured confluent primary BM-MSC for 48 hours (h), 72h and 168h. Real-time PCR was used to verify the RNA sequencing data and Western Blot analysis to confirm protein expression. Lentivirus mediated knockdown was used to target gene expression in the BM-MSC. Senescence was assessed by β-Galactosidase staining. Results from the RNA sequencing carried out to compare 10 healthy and 10 AML BM-MSC show that 1125 genes were differentially expressed, with 924 down-regulated in AML derived BM-MSC and 201 up-regulated. From this analysis, we found that CDKN1A (p21) is up-regulated in BM-MSC from AML patients (7.406 logFC) compared to BM-MSC from patients with normal bone marrow (0.382 logFC). p21 mRNA and protein expression is increased in BM-MSC when co-cultured with primary AML. Furthermore, we found that AML increased senescence β-Galactosidase staining in BM-MSC and that p21 knockdown in BM-MSC reversed the senescent phenotype. Finally, primary AML cultured on p21 knockdown BM-MSC had reduced survival compared to control BM-MSC.

**Summary/Conclusions:** We have identified that AML induces a senescent BM-MSC niche via the p21 mediated pathway which in turn promotes survival and proliferation of AML. Silencing of p21 within the BM-MSC reduces AML survival. Identifying these novel microenvironment feedback loop in AML we highlight a potential new target for future AML therapies.

**E874**

**HYPOXIA DRIVES AML PROLIFERATION IN THE TUMOR MICROENVIRONMENT THROUGH HIF1Α/MIF SIGNALLING**

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**Background:** The bone marrow microenvironment is hypoxic and furthermore hypoxia contributes to the development and maintenance of acute myeloid leukemia (AML) cells within the bone marrow microenvironment. The hypoxic state is principally maintained by members of the hypoxia-inducible factor (HIF), in particular HIF1α and its target genes, including MIF. We have previously shown that AML cells express constitutively high macrophage migration inhibitory factor (MIF) which drives IL-8 expression by the BM-MSC which in turn supports AML cell survival and proliferation (Abdul-Aziz et al, 2017).

**Aims:** The aim of the present study is to determine the role of hypoxia in regulating MIF signalling in AML.

**Methods:** Primary AML were isolated from the bone marrow and peripheral blood of AML patients following informed consent and under approval from the UK National Research Ethics Service (LRCEref07/H0310/146). Differential expression analysis of RNA sequencing data (geo ID: GSE49642) was used to correlate ox blood-derived AML cells with the expression of MIF in healthy blood. We have previously shown that AML cells express constitutively high macrophage migration inhibitory factor (MIF) which drives IL-8 expression by the BM-MSC which in turn supports AML cell survival and proliferation (Abdul-Aziz et al, 2017).

**Results:** Our results show that MIF gene expression was significantly higher in AML samples from the BM compared to those from PB. To determine if MIF is required for AML expansion, we mimicked the hypoxic conditions of the BM using CoCl2, DFO and a hypoxic chamber. We found that 1% O2, CoCl2 and DFO upregulated MIF transcription and protein expression in OCI-AML3 cell lines and in primary AML blasts. Lentiviral mediated KD of HIF1α decreased MIF expression in human AML cells and significantly reduced leukemic proliferative capacity. Moreover, KD of HIF1α in OCI-AML3 significantly increased survival of NSG mice compared to control-KD. Finally, in vivo lentiviral mediated knockdown of MIF and pharmacological targeting of MIF using ISO-1 significantly increased survival of AML xenografts.

**Summary/Conclusions:** The results reported here suggest that hypoxia significantly affects the expression of the pro-tumoural cytokine MIF in AML blasts and that this hypoxia regulated HIF1α/MIF axis supports AML blast survival in the bone marrow niche.

**E875**

**BONE MARROW ECOLOGICAL COLLAPSE IN ACUTE MYELOID LEUKAEMIA IS MEDIATED BY REMODELING OF ENDOSTEALE VESSELS**

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**Background:** Bone marrow vascular niches have been proposed to support acute myeloid leukemia (AML) growth. However, anti-angiogenic therapies do not improve patient outcome suggesting that a complex relationship between AML cells and the microenvironment influences the disease process.

**Aims:** We aim to study the complex vascular remodelling occurring during AML progression.

**Methods:** Using a murine model of AML we performed intravitral microscopy to investigate leukemia behavior in the bone marrow.

**Results:** We show AML is an invasive species causing highly localized disruption of the endostele stroma and outcompeting non-malignant cells. Particularly affected are endosteal microenvironments containing osteoblastic cells and type H endothelium, typically associated with hematopoietic stem cells (HSCs). Infiltrating leukemic endosteal cells expand, suggesting de novo niches in the spleen could potentially support extramedullary hematopoiesis in leukemia. Intravitral microscopy further revealed that the endothelium in AML is more adhesive and permissive to transendothelial migration of hematopoietic cells. Pharmacological intervention known to induce type H endothelium preserved HSC niches on endosteal areas.

**Summary/Conclusions:** Together, these data suggest that AML-induced vascular damage contributes to cell egress from the bone marrow, and that new therapeutic approaches aiming to normalize bone marrow vasculature may support normal hematopoiesis.

**E876**

**CLONAL HETEROGENEITY IN PATIENT-DERIVED XENOGRAFTS OF ADULT ACUTE MYELOID LEUKAEMIA**

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**Background:** Acute myeloid leukemia (AML) is the most common leukemia in adults. Currently, despite intensive chemotherapy and bone marrow transplantation, outcome is still dismal. In particular, therapeutic stratification remains suboptimal, which is largely attributed to the clinical and molecular heterogeneity of AML.

**Aims:** To better characterize and study this heterogeneity, we developed an in vivo model of AML using patient derived xenografts (PDx).

**Methods:** The bone marrow xenografts were established in NOD-SCID gamma null (NSG) mice. Engraftment was confirmed to be indicative of intravenous injection into the bone marrow. Cells collected from bone marrow and spleen were used to perform targeted sequencing (AmpliSeq, Thermo Fisher Scientific) and gene expression analyses (HG-U133 Plus 2.0 microarray, Affymetrix). Bone marrow cells were serially transplanted into secondary and tertiary animals. We then compared mutational and gene expression profiles of patient samples at diagnosis and corresponding PDx samples.

**Results:** The xenografts of 45 injected samples (40%) successfully engrafted into mice with a median delay of 2.5 months (range: 26-154 days). Leukaemia infiltration into bone marrow was concordant with peripheral blood and spleen infiltration. Successful engraftment was linked to younger age (50 vs 61 years, p=0.04) and elevated white blood cell counts at diagnosis (132 vs 35 G/L, p=0.001). No association was found between engraftment and karyotype or ELN classification. Relapse free survival (RFS) was worse for patients with successful PDx (0.3 vs 0.9 years, p=0.017). Despite previous reports suggesting better engraftment of AML harbouing FLT3-ITD mutations, we did not find
a preferential enrichment in the presence of FLT3-ITD mutation (9 of 18). Furthermore, we found that the mutual fractional mutation of 3 out of 4 patients harbouring a FLT3-ITD mutation enriched for this mutation in the primary PDX and then remained stable in subsequent xenotransplantations. Similarly, eight PDX with respective primary AML were analysed by next-generation sequencing (NGS) of 27 AML relevant genes. We found stable variant allele fractions (VAF) among the primary and serial PDX bone marrows and spleens for 50 mutations (6% of all mutations on average per patient); except for DNMT3A and NRAS mutations, which were lost each for one AML sample at the secondary PDX. Concordantly, gene expression profiles were stable between primary patient sample and serial PDX samples up to the tertiary xenograft.

Summary/Conclusions: NSG mice xenotransplantation may be a clinical relevant model for in vivo studies of clonal heterogeneity in AML and may be used as preclinical model to test novel therapies.

E877 COSTIMULATION INCREASES INTRACELLULAR SIGNALING IN BITE® ANTIBODY CONSTRUCT MEDIATED T-CELL ACTIVATION
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Background: The CD19/CD3 BiTE® antibody construct, blinatumomab, has been approved in Ph−, relapsed/refractory B-cell precursor Acute Lymphoblastic Leukemia (ALL) and Acute Myeloid Leukemia (AML) without co-stimulatory molecules (B33). In contrast, MOLM-13 cells were completely lysed (% specific lysis relative to control B33 vs 80.7±16.1, n=3). We next analysed intracellular Akt and Erk phosphorylation levels of T cells after stimulation with AMG 330 or a control BITE® antibody construct (cBITE®) and CD19/CD3 vs CD3/CD28 antibodies semi as positive control. In the presence of target cells, AMG 330 induced significantly lower Akt and Erk phosphorylation (mean% phosphorylated (p)Akt and pErk 7.9 and 7.6, n=3) compared to crosslinked CD3/CD28 antibodies (mean% pAkt and pErk 43.0 and 34.6). However, the combination of AMG 330 and CD28 increased the amount of phosphorylated proteins (mean% pAkt and pErk 11.6 and 11.1), but not to the level achieved by CD3/CD28 stimulation. In the absence of target cells, no Akt phosphorylation was observed upon incubation with AMG 330, suggesting a highly target cell dependent T-cell activation (mean% pAkt with vs without target cells: 0.8 vs 7.9).

E878 ESTABLISHING SINGLE CELL WHOLE EXOME SEQUENCING ANALYSIS AS A DISCOVERY TOOL IN NPM1/FLT3 POSITIVE PEDIATRIC ACUTE MYELOID LEUKEMIA
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Background: AML is a rare hematological disorder in children and adolescents caused by distinct genetic aberrations, which are relevant for leukemogenesis, prognosis and therapy. Although major players in the molecular landscape and clonal evolution of AML have been identified in adults, it remains a major technical challenge to genetically characterize the few leukemic stem cells (LSCs) cells against a noisy background of AML blasts and normal hematopoietic cells.

Aims: The aim of this study was to establish a simple workflow for reliable analysis of single LSCs in pediatric patients with AML, where often limited research material is available.

Methods: For three pediatric AML patients with mutations in the genes NPM1 and/or FLT3, we performed single cell sorting for CD34+ CD38- AML blasts by FACS and subsequently whole genome amplification (WGA) using the REPLiG single cell DNA kit (Qiagen). The amplified single cell DNA and additional DNA of the corresponding bulk bone marrow was analysed by exome sequencing (WES). Bulk DNA was additionally evaluated by an amplicon-based sequencing approach targeting 54 genes (TruSight Myeloid Panel, Illumina) associated with myeloid malignancies.

Results: The analysis revealed that the median read coverage obtained in the WES of the five DNAs amplified from the single CD34+ CD38- cells and in the corresponding bulk DNAs from the bone marrow of all three patients was comparable for three out of the five single cells. For three amplified single cell genomes, between 92 and 98% of all reads could be mapped to the human genome, however the median coverage for the two failed single cells was 0. For validation of the WGA quality from single LSC DNA, data from 50 out of 54 genes analyzed by both sequencing approaches, WES and TSM Panel, were available for all three patients. The majority of variants detected in the WES bulk data could consistently be found at a comparable variant frequency in the TSM panel data. The variant frequencies in the single cell data from WES were more variable and more variants could not be detected in the TSM panel data derived from bulk DNA. We were able to detect n=79 (66%) out of n=121 somatic variants (SNVs, InDels) present in the patients’ AML blasts with all three sequencing approaches. WES readily identified n=103 (85.1%) and n=93 (78.9%) of all 121 variants in the bulk and single cell DNA, respectively. Only n=4 (3.3%) variants were not detected by WES at all. We were able to retrace the NPM1 and FLT3 mutations for each of the three patients in the targeted sequencing approach. However the NPM1 mutations and one FLT3 ITD could not reliably be called in the WES approach due to insufficient coverage.

Summary/Conclusions: In summary, WES of amplified single cell DNA is an excellent discovery tool also in pediatric AML for detecting unique changes in potential LSCs that should be validated by targeted sequencing approach with sufficient read counts for finding of rare events.

E879 RAF KINASE INHIBITOR PROTEIN IS INVOLVED IN THE DEVELOPMENT OF MYELOID SARCOMA
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Background: Myeloid sarcoma (MS) is a subgroup of acute myeloid leukemia (AML), where leukemic invades non-hematopoietic tissues and form solid tumours. It may occur as isolated event or simultaneously with leukemia infiltration of the bone marrow (BM). Loss of RAF kinase inhibitor protein (RKIP), a negative regulator of Ras signaling, has recently been described as a frequent event in AML and to be functionally involved in leukemogenesis. Although RKIP has been shown to inhibit the formation of metastases in solid tumors previously, its role in the development of MS is currently unknown.

In this study, we aimed to delineate the role of the metastasis-suppressor RKIP in the development of MS.

Methods: RKIP protein and mRNA expression was evaluated in formalin-fixed paraffin-embedded biopsies of MS and BM by immunohistochemistry and quantitative real-time PCR (qPCR). Sequence analysis of MS biopsies defined as MS were carried out by targeted next generation sequencing (NGS). For functional assays, both RKIP overexpression and knockdown was performed in THP-1 AML cells by lentiviral transduction of a FLAG-tagged RKIP expression construct and by RKIP shRNA, respectively. Subsequently, these cells were tested in migration and invasion assays using transwell-methodology.

Results: This study comprised 14 patients with MS (MS-group) and 14 patients with AML without any evidence of extramedullary involvement (BM-AML group). Of the 14 cases within the MS-group, MS occurred as isolated event in three cases and concomitantly with systemic AML in eleven cases. Both groups were assessed in this matched and clinical as well as laboratory values were comparable between them. Most importantly, however, when we measured the protein expression of RKIP in leukemic tissues of these patients (MS biopsies in the MS-group and leukemic BM biopsies in the BM-AML group), we observed a
significant increase of specimens exhibiting loss of RKIP expression in the MS-group (7/14 vs 1/14, P=0.0329). Interestingly, RKIP loss in MS specimens of cases with concomitant systemic AML was also present in the corresponding leukemic BM samples, thereby excluding a geographical clonal heterogeneity during MS formation in respect to RKIP expression. We then analyzed RKIP mRNA levels by qPCR and observed that RKIP loss correlated with decreased expression of RKIP (P=0.041). To gain more insight into the molecular landscape of MS patients with and without RKIP loss, we performed NGS of 39 genes that are recurrently mutated in AML. Interestingly, five out of six (83%) MS patients with RKIP loss demonstrated mutation(s) affecting the RAS-pathway, suggesting a potential functional synergism between these events. Consequently, we performed an overexpression and knockdown of RKIP in the RAS-mutated THP-1 AML cell line and subsequently studied these cells in functional migration and invasion assays. Importantly, RKIP knockdown increased both migration and invasion, thereby indicating a role of RKIP in the development of this condition.

E880

INHIBITING MIR-10A OVERCOMES CYTARABINE-RESISTANCE IN ACUTE MYELOID LEUKAEMIA

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Background: Chemoresistance is the principle cause of treatment failure in acute myeloid leukaemia (AML) despite a promising response to induction chemotherapy. Emerging evidence suggests the roles of autophagy, a self-eating process contributing to chemoresistance of leukaemia cells. We previously demonstrated that miR-10a, highly expressed in a subgroup of AML harbouring NUP98-HSPC101 mutations, promotes cell survival by inhibiting non-canonical cell death pathway, suggesting its function in autophagy and thus chemoresistance in AML.

Aims: We aim to demonstrate evidence that miR-10a, a regulator of autophagy, plays important roles in chemoresistance in acute myeloid leukaemia.

Methods: Apoptosis and proliferation in miR-10a inhibited and overexpressed leukaemia cells after cytarabine treatment was measured by Annexin V binding and MTT assay. Autophagy was measured by monitoring the levels of LC3I/LC3II proteins, autophagy-related proteins via Western Blotting and monodansyl-cavardine (MDC) staining (flow cytometry).

Results: First, we observed a decreased expression of miR-10a in the leukaemia cells after the exposure to stress induced by serum starvation. Overexpressing miR-10a in miR-10a-low MV4-11 cells decreased apoptosis induced by nutrient starvation and resulted in the resistance to cytarabine. In contrast, its inhibition in OCI-AML3 cells, which express high miR-10a constitutively, resulted in the induction of apoptosis and increased chemosensitivity towards cytarabine. miR-10a was shown to directly downregulate key members of the p53-mediated tumour suppressor gene network, including the CDKN1A (p21) inhibitor Transcription Factor AP2-gamma (TFAP2C). The inhibition of either miR-10a itself or CDKN1A by siRNA treatment inhibited apoptosis induced by serum starvation, treatment with autophagy inducer, mg132 or p35 stabiliser, Nutlin3a.

Summary/Conclusions: The data suggests miR-10a as an important regulator of autophagy and a modulator p53-p21 tumour suppressor signalling axis in subtypes of AML. It also emphasizes the significance of autophagy in chemoresistance in AML, supporting the targeting of the autophagy pathway as a potential therapeutic approach for AML.

E882

DYSREGULATION IN KEY REGULATOR GENES OF AUTOPHAGY AS A MECHANISM OF THERAPY RESISTANCE AND POOR PROGNOSIS IN ACUTE MYELOID LEUKAEMIA (AML): RESULTS FROM MICROARRAY ANALYSIS ON 148 PATIENTS

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Background: To date, there are no clear evidences if autophagy can lead to therapy resistance or favor apoptosis in cancer. Autophagy can function as a pro-apoptotic mechanism, or can improve stresses survival clearing damaged mitochondria and proteins accumulation. Levels and activity of pro-apoptotic and anti-apoptotic proteins, particularly BCL-2 and p53, high levels of CAMP, and a complex made by PINK/PARK could play as fulcrum of this yin and yang effect of autophagy.

Aims: Our study aims to define the role of PI3P pathways in AML, and to establish if autophagy could reduce the patients’ chance to respond to induction, and to worsen OS.

Methods: We analyzed 148 consecutively newly diagnosed non M3 AML patients treated with induction chemotherapy regimens containing at least one dose of anthracycline. We screened all patients for TP53, FLT3, NPM1 mutations. In all
Background: MSI is the addition or loss of bases within repetitive DNA sequences. MSI is a form of microsatellite instability, which is a genomic instability characterized by the accumulation of MSI mutations within the genome. MSI is associated with poor survival in AML patients.

Methods: We analyzed 1,394 AML patients for MSI using a combination of NGS and microsatellite analysis. The NGS results were confirmed by microsatellite analysis using a panel of 18 markers. The AML group was divided into three subgroups: group 1 genes with CN gain, group 2 genes with CN loss, and group 3 genes with CN loss and MSI.

Results: A subset of AML patients had MSI, with 10% of the patients having MSI-positive tumors. The MSI-positive tumors had higher levels of heterozygosity and were more likely to have alterations in the genes involved in the DNA damage response and repair pathways. The MSI-positive tumors also had a higher frequency of mutations in genes involved in cell cycle regulation and DNA repair. The MSI-positive tumors were more likely to have a worse overall survival compared to MSI-negative tumors.

Summary/Conclusions: Our work identifies a subset of AML patients with MSI, which may have implications for targeted therapy and prognosis. Further studies are needed to determine the clinical relevance of MSI in AML.
Aims: To identify key downstream mediators of SYK signaling in AML respon-
sible for differentiation block, proliferation and leukemic stem cell (LSC) main-
tenance.

Methods: AML cell lines (KG1, MOLM14) or bone marrow primary AML blasts, were incubated 24h with R406 (1uM, 4 uM) or vehicle. Activity of SYK, ERK, STAT5 was assessed by western blot and/or intracellular phospho-flow. Prolif-
eration was assessed by using the TruSight Myeloid Sequencing Panel (Illumi-
a) to analyze 42 target genes or hotspots known to be mutated in AML or other hematologic neoplasms.

Results: In 48 biCEBPA and 32 moCEBPA we found mutations in 20 and 26 different genes respectively. MoCEBPA pts had significantly more additional mutations compared to biCEBPA pts (mean: 3.9±1.7 vs 2.2±1.5; p<.001). We also compared the mutational profile of moCEBPA and biCEBPA pts with a cohort of 10 wildtype (wt) AML pts. In biCEBPA pts, mutations in ≥5% were significantly associated with one or more groups. We confirmed the mutual exclusiveness of biCEBPA and NPM1 and the association between GATA2 and biCEBPA (35.4%). TET2 was frequently mutated in both mo- (43.8%) and biCEBPA pts (41.7%), but not in wtCEBPA (16.3%; mo-vs wtCEB-
PA p<.001; bi-vs wtCEBPA p=.001). Mutations in TKD1 or TKD2 of FLT3 were frequently identified in moCEBPA (25%) and wtCEBPA (17.8%) but not in biCEBPA pts (2.1%). The FLT3-TKD1/2 mutation frequency in biCEBPA sig-
ificantly differs from moCEBPA (p=0.002) and wtCEBPA (p=.004). There was a significant difference in the frequency of FLT3-TKD in biCEBPA (20.8%) vs wtCEBPA (0.4%) (p<.001) but not in comparison to moCEBPA (43.8%). IDH2 was found mutated only in wtCEBPA (21.6%) and moCEBPA (18.8%). In 48.8% of wtCEBPA pts DNMT3A was mutated, this significantly differs from biCEBPA pts (14.3%; p<.001) but not from moCEBPA patients (28.1%). C3RF3 was frequently mutated only in biCEBPA (10.4%) but not in wtCEBPA (0.35%; p<.001) or moCEBPA (3.1%; ns). Stag2 was associated with moCEBPA (25%), while STAG2 mutations were significantly less frequent in biCEBPA (6.3%; p<.001) and wtCEBPA pts (6.27%; p=.002). TET2 mutations had a negative prognostic impact on overall survival (OS) in biCEBPA pts, but not in wtCEBPA or moCEBPA pts (17.8% vs 2.1% vs 6.1%; p=.03; 95% CI: 1.2-8.2; p=.023). The number of additional mutations in biCEBPA pts was significantly higher (RFS) and cumulative incidence of relapse (CIR) was not different depending on TET2 status. In biCEBPA pts we also evaluated the clinical impact of GATA2 mutations. For 30 of 48 biCEBPA pts survival data was available, 15 of these pts had GATA2 mutations. We found a significant difference with respect to RFS (p=0.216), OS (p=0.479) and CIR (p=0.599). In a combined analysis, the GATA2mut and TET2wt genotype was associated with a lower relapse risk and a trend towards a higher RFS compared to the GATA2wt and TET2wt genotype.

Summary/Conclusions: We can confirm the distribution of the 91 patients in the 3 relapse-risk groups was: 28 in low-risk group (21.6%), 48 in intermediate-risk (53%) and 15 in high-risk (16%). We found that SYK inhibition obviates differentiation block, proliferation and leukemic stem cell (LSC) mainten-
ance.

Methods: AML cell lines (KG1, MOLM14) or bone marrow primary AML blasts, were incubated 24h with R406 (1uM, 4 uM) or vehicle. Activity of SYK, ERK, STAT5 was assessed by western blot and/or intracellular phospho-flow. Prolif-
eration was assessed by using the TruSight Myeloid Sequencing Panel (Illumi-
a) to analyze 42 target genes or hotspots known to be mutated in AML or other hematologic neoplasms.

Results: In 48 biCEBPA and 32 moCEBPA we found mutations in 20 and 26 different genes respectively. MoCEBPA pts had significantly more additional mutations compared to biCEBPA pts (mean: 3.9±1.7 vs 2.2±1.5; p<.001). We also compared the mutational profile of moCEBPA and biCEBPA pts with a cohort of 10 wildtype (wt) AML pts. In biCEBPA pts, mutations in ≥5% were significantly associated with one or more groups. We confirmed the mutual exclusiveness of biCEBPA and NPM1 and the association between GATA2 and biCEBPA (35.4%). TET2 was frequently mutated in both mo- (43.8%) and biCEBPA pts (41.7%), but not in wtCEBPA (16.3%; mo-vs wtCEB-
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ificantly differs from moCEBPA (p=0.002) and wtCEBPA (p=.004). There was a significant difference in the frequency of FLT3-TKD in biCEBPA (20.8%) vs wtCEBPA (0.4%) (p<.001) but not in comparison to moCEBPA (43.8%). IDH2 was found mutated only in wtCEBPA (21.6%) and moCEBPA (18.8%). In 48.8% of wtCEBPA pts DNMT3A was mutated, this significantly differs from biCEBPA pts (14.3%; p<.001) but not from moCEBPA patients (28.1%). C3RF3 was frequently mutated only in biCEBPA (10.4%) but not in wtCEBPA (0.35%; p<.001) or moCEBPA (3.1%; ns). Stag2 was associated with moCEBPA (25%), while STAG2 mutations were significantly less frequent in biCEBPA (6.3%; p<.001) and wtCEBPA pts (6.27%; p=.002). TET2 mutations had a negative prognostic impact on overall survival (OS) in biCEBPA pts, but not in wtCEBPA or moCEBPA pts (17.8% vs 2.1% vs 6.1%; p=.03; 95% CI: 1.2-8.2; p=.023). The number of additional mutations in biCEBPA pts was significantly higher (RFS) and cumulative incidence of relapse (CIR) was not different depending on TET2 status. In biCEBPA pts we also evaluated the clinical impact of GATA2 mutations. For 30 of 48 biCEBPA pts survival data was available, 15 of these pts had GATA2 mutations. We found a significant difference with respect to RFS (p=0.216), OS (p=0.479) and CIR (p=0.599). In a combined analysis, the GATA2mut and TET2wt genotype was associated with a lower relapse risk and a trend towards a higher RFS compared to the GATA2wt and TET2wt genotype.

Summary/Conclusions: We can confirm the distribution of the 91 patients in the 3 relapse-risk groups was: 28 in low-risk group (21.6%), 48 in intermediate-risk (53%) and 15 in high-risk (16%).
found 150 mutations in 31 genes, in 73 out of the 91 patients included (a median of 1 mutation per patient (range: 0-5) with a mean read depth of 1036x. Eighteen patients remained wild-type for all analyzed genes (Figure 1).One only of this patients suffered relapse (5%). In the global series, no single mutation or functional category showed an association with clinical variables or prognostic impact in terms of overall survival or relapse free survival (RFS). There were no differences in the mean number of mutations per patient in each risk APL group (p=0.05). Patients who lack mutations belonged to the intermediate (13/48, 27%) and low risk (4/28, 14%) groups, except for only one patient (1/15, 6%) in high-risk group. FLT3 was the most frequently affected gene in high risk APL subgroup (10 out of 15): 8 patients carried an FLT3-ITD mutation and 2 patients had amino acid substitutions at codon 835. Seven patients assigned to intermediate-risk relapsed (7/38, 18%). All but one carried mutations that have been reported as unfavorable in AML (FLT3, PTEN, ASXL1, CUX1 and WT1). By contrast, patients who remain in complete remission in this group, lack mutations with a greater frequency (12/31, 39%). Finally, within the low-risk group 3 patients suffered relapse (3/27, 11.5%) and all of them presented missense mutations in the Ras domain of NRAS at diagnosis (p.Ser65Arg & p.Gln61Arg). Therefore, we could identify a small subgroup of patients at a very high risk of relapse (RFS at 5 years, 25% vs 100%, p<0.001).

Figure 1.

Table 1.

Summary/Conclusions: In summary, the present study shows that the mutational status of NRAS and FLT3 genes could be used as genetic markers for prognosis of APL, especially in the intermediate and low-risk groups, allowing a more accurate patient risk classification. Our data suggests the need to search for new mutations required for progression in APL, in order to benefit from a more accurate patient risk classification. In addition status of NRAS and FLT3 genes could be used as genetic markers for very high risk of relapse (RFS at 5 years, 25% vs 100%, p<0.001).

E888
ANALYSIS OF THE PD-1/PD-L1 AXIS POINTS TO ASSOCIATION OF UNFAVORABLE RECURRENT MUTATIONS WITH PD-L1 EXPRESSION IN AML

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Background: Programmed death ligand-1 (PD-L1) is regulated through miR-34a molecules in AML patients. Moreover, Cortez et al. for the first time identified novel, complete mechanism of PD-L1 regulation by p53 via miR-34a in non-small cell lung cancer (NSCLC).

Aims: In this study, our comprehensive analyses of PDCD1 (PD-1), CD274 (PD-L1), TP53 and miR-34a expression in AML patients shed new light on the complex regulation of PD-1/PD-L1 axis during development of this disease.

Methods: We performed analysis of TP53, CD274 and miR-34a expression in 197 AML patients available from The Cancer Genome Atlas (TCGA) database. Moreover, we assessed mRNA expression of PDCD1 in independent cohort of 54 AML, 62 MDS and 8 s-AML patients samples using qRT-PCR method. For miRNA analysis, CD33+ cells from 29 AML patients were isolated and compared to 29 healthy volunteers (HV). TCGA data analysis showed that CD274 expression was elevated in group with TP53 mutations compared to unmutilated TP53 group (p<0.001). We also found negative correlation of TP53 and miR-34a expression with CD274 expression (p=0.02 and p=0.005, respectively). The expression of miR-34a tended to be elevated in group with high expression of TP53 compared to group with low TP53 expresion (p=0.17). We have not found any differences in CD274 expression between groups with or without following mutations: IDH1, TET2, RUNX1, NRAS, CEBPA, PTPN11, KIT, KRAS, FLT3, DNM3, NPM1 and IDH2. Patients with more than 4 recurrent mutations were characterized with higher expression of CD274 compared to group of patients with 0-3 recurrent mutations. We also found that patients with >14 of all mutations had elevated expression of CD274 compared to group 0-13 mutations (p=0.06). We observed significant differences in PDCD1 expression level regarding to PD-1.1.5 polymorphism. Moreover, analysis of a PD-1.1.3 polymorphism in HVs and MDS groups revealed that genotype CC was associated with nearly fivefold lower risk of disease (OR=4.93, p=0.009). We observed significant differences in OS in AML patients in case of presence of certain genotypes of PD-1.1.6. Genotype AA was significant associated with higher risk of shorter OS compared to the rest of the genotypes (58 vs 333 days, HR=35; p=0.001).

Summary/Conclusions: Our analyses indicate that p53 might specifically modulate the tumor immune response by regulating PD-L1 via miR-34a which directly binds to the PD-L1 3’ UTR and blocks its expression. Moreover, we found that high CD274 expression is associated with the higher numbers of recurrent and all mutations as well as poor cyogenetic and molecular risk groups of AML patients. We found significant differences in PDCD1 expression in AML patients compared to HVs that might indicate deregulation of a signal transduction through the PD-1/PD-1L axis. While our SNP analysis in AML patients suggested a prognostic impact of PD-1.6 polymorphism, further studies are warranted to evaluate the impact of the PD-1/PD-1L Axis in AML.

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E889
DISSECTING THE DYNAMICS OF SINGLE-TUMOR-CELL-LINEAGES THAT UNDERPIN RELAPSE OF AML

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Background: Cancers kill primarily via disease recurrences after transient treatment responses. The emergence of therapy-resistant tumor escape variants is fueled by intra-tumor heterogeneity, underpinned by interference and Darwinian evolution across continuously developing sub-clones in the residual tumor. Several non-genetic factors add significant variation, on top of the diver-...
and detected at diagnosis and at follow-up (after induction, first consolidation or follow-up: specific region of the four most frequent alterations at diagnosis (Samples at diagnosis sample; From the 32 genes, we use specific primers to amplify the mentioned. We developed a custom-targeted sequencing panel of 32 genes patients treated according to the appropriate treatment regimens. However, some patients who achieved a negative MRD become to relapse and several MRD+ patients have minimal quantitative impact (i.e. yields similar cell number per regrowth). Importantly, this cell type is stochastically selected by the reverse of resistance to chemotherapy re-treatment, which the DOX-containing treatment regimens potentely induced in the absence of DCT.

Summary/Conclusions: The development of curative treatment combinations requires deep understanding of how non-genetic factors synergize with cancer genetics to drive intra-tumor heterogeneity, which is key for tumor escape/disease recurrence. Our detailed analyses of the heterogeneous dynamics among single-lineage cells in AML, following different treatment regimens with apparently similar global impact, represent an important step in dissecting kinship-dependent aspects that go beyond the genetic level. Critically, these studies directly provide the rationale for combining standard chemotherapy with administration of hypomethylating drugs to target AML. The mechanism is prevention of the development of chemotherapy resistance (mediated by selective release of a specific set of predetermined sub-lineages) - by partially randomizing which sub-lineages that regrew - specifically making replicates more divergent from each other, indicating a more stochastic selection of the cells emerging when DCT had been added to the respective chemotherapy regimens. Practically, this cell type is stochastically selected by the reverse of resistance to chemotherapy re-treatment, which the DOX-containing treatment regimens potentely induced in the absence of DCT.

Summary/Conclusions: High-throughput NGS is a technique with the capacity to measure, identify and classified MRD levels. In fact, NGS MRD evaluation has a better DFS and OS prediction than other traditional methods. Implementation of NGS technique on MRD detection could help to anticipate to disease progression.

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E892
THE ROLE OF MYELOID-DERIVED SUPPRESSOR CELLS-LIKE BLASTS WHICH SUPPRESS T CELL PROLIFERATION IN LEUKEMIC CELL GROWTH
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Background: Myeloid-derived suppressor cells have an ability to suppress T-cell function and have been known to facilitate tumor growth. We elucidated the immune suppressive function of leukemic blasts which resembled MDSC phenotype and their role in the growth of leukemic cells.

Aims: We elucidated the immune suppressive function of leukemic blasts which resembled MDSC phenotype and their role in the growth of leukemic cells.

Methods: CD11b+CD33+HLA-DR blast (MDSC like blast) were isolated using flow-cytometry from bone marrow mononuclear cells of primary acute myeloid leukemia (AML) patient samples. CD14, CD15, Arg1 and iNOS expression were checked by flow-cytometry to identify the phenotype of MDSC like blast. To evaluate the ability of MDSC like blasts to suppress T cell proliferation, CD8 T cells from healthy donor and MDSC like blasts were co-cultured with the ratio of 1:1 with/without phytohemagglutinin-A (10ug/mL). T-cell proliferation was measured by carboxyfluorescein diacetate succinimidyl ester dilution assay after 3 days of culture. Then, various leukemic cell lines were co-cultured with jurkat T cells and/or MDSC like blasts at a leukemic cell line:jurkat T cell: MDSC like blast ratio of 4:4:1. The effect of jurkat T cells and MDSC like blasts on the proliferation of leukemic cells was assessed by the CCK-8 assay after 1 and 3 days of culture.

Results: MDSCs like blast can be divided into two subtypes, monocyteic sub-group expressing CD14 and granulocytic subgroup expressing CD15, and CD14 expression was more frequent than CD15 (67.5% vs 39.3%). MDSC- like blasts showed higher expression of ARG1 (77.1% vs 38.5%, P<0.001) and iNOS (33.0% vs 1.1%, P=0.0001) compared to non-MDSC-like blasts. CD8 T cell proliferation induced by PHA was significantly suppressed when co-cultured with MDSC-like blasts compared to without them. Among the various leukemic cell lines, the proliferation of NB4 cells were significantly suppressed when co-cultured with jurkat T cells on day 3 (NB4 23.49±6.26% of control, NB4-jurkat 12.62±3.92%, P<0.01). The decreased proliferation of NB4 cells was partially recovered after 3 days of co-culture with MDSC-like blasts (NB4-jurkat 12.62±3.92%, NB4+jurkat+MDSC like blast 18.71±6.19, P=0.022).
Summary/Conclusions: CD11b*CD33*HLA-DR* MDSC-like blasts subpopulation which expressed the INOS and Arg1 existed in AML, and showed ability to suppress the T cell proliferation. MDSC-like blasts partially restored the suppressed leukemic cell growth of NB4 cells by jurkat cells. MDSC-like blasts might play a certain role in immune-escape mechanism of AML.

E893

GENERATION OF NEW CELLULAR MODELS FOR THE STUDY OF PEDIATRIC NON DOWN SYNDROME ACUTE MEGAKARYOBLASTIC LEUKEMIA BASED ON HUMAN PLURIPOTENT STEM CELLS

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Background: Acute megakaryoblastic leukaemia (AMKL) is a rare and complex type of Acute Myeloid Leukaemia (AML), more frequent in children than in adults, characterized by the accumulation of immature megakaryoblasts and thrombocytopenia. Paediatric AMKLs are classified in Down Syndrome AMKL (DS-AMKL) with a good prognosis; and AMKL non-related to Down Syndrome (non-DS-AMKL), a more aggressive disease with a mortality rate close to 80%. There is a limited amount of research done on infant non-DS AMKL due to its low prevalence and the scarcity of the gene mutations linked to it. Among the genetic alterations found in non-DS-AMKL, approximately half of the patients carry the chromosomal translocations t(1;22) and t(11;12), that generate the fusion proteins RBM15-MKL1 and NUP98-JARD1 respectively, and the inversion of chromosome 16, that originates the fusion protein CBFA2T3-Glut1. Therefore, MDSC-like blasts might play a certain role in immune-escape mechanism of AML. Furthermore, unidirectional GSPs provided bidirectional coverage of a BCR-ABL1 fusion, which was detected with reads originating from ABL1 as well as BCR. Using our optimized bioinformatics algorithm and the VariantPlex assay, we accurately and reliably detected IDTs of varying sizes and insertion points, with simultaneous point mutation detection, in AML-positive blood samples. Furthermore, we show multiple mutations in various AML-positive sample types, including mutations in CEBPα. Finally, MBCs used in AMP enabled NGS-based expression profiling for identification of Diffuse Large B Cell Lymphoma subtypes in a small cohort of samples.

Aims: It is essential to establish new human models to provide enough biological material for functional and molecular studies. As the genetic alterations that drive infant leukaemia occur in the developing fetus, we propose that hematopoietic progenitor cells (hPSCs) are ideal models to study non-DS AMKL, as these cells allow us to mimic human embryonic hematopoietic development. In this project, we aim to use human hPSCs expressing non-DS-AMKL-associated fusion oncogenes as cellular models for this leukaemia, to study the molecular and cellular pathways involved in the development of pediatric non-DS AMKL.

Methods: Generation of human models of non-DS AMKL using hPSCs: 1. Generation of hPSCs with the oncogenic fusion proteins RBM15-MKL1, CBFA2T3-GLIS2 and NUP98-JARD1 using transduction with lentiviral vectors. 2. Generation of hPSCs with the chromosomal translocations t(1;22)(p13;q13) RBM15-MKL1 and t(11;12)(p15;p13) NUP98-JARD1 using the CRISPR/Cas9 system. 3. Non-DS-AMKL hPSC cell lines are generated, we confirm that they preserve their pluripotency by checking expression of pluripotency markers by flow cytometry and PCR. We also confirm their ability to differentiate into the three germ layers forming embryoid bodies. Using an in vitro differentiation assay, we confirm that hPSCs are able to differentiate into megakaryoblasts and thrombocytes. We use colony-forming assays (CFU) to determine the generation and functionality of hematopoietic progenitors.

Results: Here we report the generation and characterization of human non-DS AMKL hematopoietic progenitor cells expressing oncogenic fusion proteins RBM15-MKL1, NUP98-JARD1 and CBFA2T3-GLIS2.

Summary/Conclusions: These models will serve as platforms to discover and understand the cellular and molecular alterations caused by these oncogenes, and their impact in the generation of hematopoietic cells during development. With this information we will have a better understanding of the origin and development of paediatric non-DS AMKL, so we will be able to design new therapeutic approaches for these children.

E894

CHARACTERIZATION OF HEMATOLOGIC MALIGNANCIES WITH ANCHORED MULTIPLEX PCR AND NEXT-GENERATION SEQUENCING

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Background: Acute megakaryoblastic leukaemia (AMKL) is a rare and complex type of Acute Myeloid Leukaemia (AML), more frequent in children than in adults, characterized by the accumulation of immature megakaryoblasts and thrombocytopenia. Paediatric AMKLs are classified in Down Syndrome AMKL (DS-AMKL) with a good prognosis; and AMKL non-related to Down Syndrome (non-DS-AMKL), a more aggressive disease with a mortality rate close to 80%. There is a limited amount of research done on infant non-DS AMKL due to its low prevalence and the scarcity of the gene mutations linked to it. Among the genetic alterations found in non-DS-AMKL, approximately half of the patients carry the chromosomal translocations t(1;22) and t(11;12), that generate the fusion proteins RBM15-MKL1 and NUP98-JARD1 respectively, and the inversion of chromosome 16, that originates the fusion protein CBFA2T3-Glut1. Therefore, MDSC-like blasts partially restored the suppression of T cell proliferation. MDSC-like blasts expressed leukemic cell growth of NB4 cells by jurkat cells. MDSC-like blasts partially restored the suppressed leukemic cell growth of NB4 cells by jurkat cells. MDSC-like blasts might play a certain role in immune-escape mechanism of AML. Furthermore, unidirectional GSPs provided bidirectional coverage of a BCR-ABL1 fusion, which was detected with reads originating from ABL1 as well as BCR. Using our optimized bioinformatics algorithm and the VariantPlex assay, we accurately and reliably detected IDTs of varying sizes and insertion points, with simultaneous point mutation detection, in AML-positive blood samples. Furthermore, we show multiple mutations in various AML-positive sample types, including mutations in CEBPα. Finally, MBCs used in AMP enabled NGS-based expression profiling for identification of Diffuse Large B Cell Lymphoma subtypes in a small cohort of samples.

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Results: Here we report the generation and characterization of human non-DS AMKL hematopoietic progenitor cells expressing oncogenic fusion proteins RBM15-MKL1, NUP98-JARD1 and CBFA2T3-GLIS2.

Summary/Conclusions: These models will serve as platforms to discover and understand the cellular and molecular alterations caused by these oncogenes, and their impact in the generation of hematopoietic cells during development. With this information we will have a better understanding of the origin and development of paediatric non-DS AMKL, so we will be able to design new therapeutic approaches for these children.

Aims: Our goal was to develop AMP-based NGS assays to simultaneously detect multiple mutation types from DNA and RNA, as well as relative gene expression levels and copy number alterations (CNAs). In particular, we sought to develop methods to detect novel gene fusions, internal tandem duplications (ITDs) and mutations in CEBPα.

Methods: We developed AMP-based PrimerZap™ and FusionPlex™ assays to enable NGS-based detection of mutations from DNA and RNA, respectively. Open-ended amplification permits identification of novel gene fusions with FusionPlex and complex mutation types such as ITDs with VariantPlex assays. MBC adapters ligated to RNA and DNA fragments prior to amplification enable relative gene expression and CNA analysis.

Results: We show instances of gene fusion detection from open-ended amplification of hPSCs, including RBM15-MKL1. We also show instances of relative gene expression and CNA analysis. We show instances of gene fusion detection from open-ended amplification of hPSCs, including RBM15-MKL1. We also show instances of relative gene expression and CNA analysis. We show instances of gene fusion detection from open-ended amplification of hPSCs, including RBM15-MKL1. We also show instances of relative gene expression and CNA analysis. We show instances of gene fusion detection from open-ended amplification of hPSCs, including RBM15-MKL1. We also show instances of relative gene expression and CNA analysis.
mutations. Moreover, ASXL1 mutations were detected in 3 of 12 patients with aberrations detected with cytogenetics (25%), 2/9 (22%) with trisomy 13, 2/11 (18%) with t(9;22) and only 1 of 22 patients with t(15;17). Multivariate logistic regression showed that independent predictors of the presence of ASXL1 mutations were older age (OR 1.43 per decade, 95% CI 1.13-1.79), chromosome 11 aberrations (OR 2.69, 95% CI 1.09-6.63), and sec-AML (OR 4.44, 95% CI 2.3-8.57), whereas as -7/del(7q) or -5/del(5q) predicted for lower frequency (OR 0.32, 95% CI 0.13-0.75).

Summary/Conclusions: Our results support the association of ASXL1 mutations in AML with advancing age and sec-AML. Association with trisomy 8 did not retain significance in multivariate analysis. Chromosome 11 aberrations emerged as a strong independent predictor. Despite the strong link with secondary AML (majority of cases post MDS), our data show inverse relationship with -7/del(7q) or -5/del(5q). In addition, ASXL1 mutations were not positively associated with MDS-related cytogenetic abnormalities, complex or monosomalous karyotypes.

E896
Abstract withdrawn.

E897
A COMPREHENSIVE DNA TEST FOR THE DETECTION OF TRANSLOCATIONS IN ACUTE LEUKEMIA
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Background: Patients with acute leukemias carry a wide range of chromosomal abnormalities, which affect their prognosis and treatment options. Currently, over 500 different translocations are reported to be involved in the disease progression. Traditional methods to detect chromosomal abnormalities involve a combination of techniques such as karyotyping, FISH, array and RT-PCR. However, these methods are laborious and at times inadequate. Targeted Locus Amplification (TLA), a new targeted next generation sequencing technology can overcome these shortcomings. It is based on proximity ligation (crosslinking) of DNA and outward oriented probes for enrichment and can therefore identify chromosomal translocation partners regardless of their identities.

Aims: Here we present a TLA multiplex panel in combination with next generation sequencing as a first tier screening tool in detecting translocations in acute leukemias.

Methods: A multiplex TLA panel was designed using primer sets covering known break-point regions of the 17 most frequently reported genes involved in acute leukemia’s. TLA was performed on five different cell lines carrying translocations detectable by our panel. [t(12;21), t(6;11), t(11;19), t(8;13), t(6;9), t(17;19)]. Various combinations of cell line mixtures in multiple dilution series were used to determine the specificity and sensitivity of the panel, and to set sample quality thresholds during analysis. Samples were processed using standard TLA protocol (de Vree et al., 2014). Targets were enriched by PCR amplification from the multiplex panel and subjected for sequencing on Illumina Nextseq 500. To facilitate an easy analysis workflow a semi-automated data analysis was developed. This includes a quality control step, labelling samples with no coverages at the anchor regions after filtering at more than half the number of target regions as failed. These were not interpreted. Only peaks outside other anchor regions were considered as false positive peaks. Peaks present in other anchor regions were interpreted as possible artefacts and labelled as needing extra confirmation. In these series until now, up to 10% aberrant cells were detected with no false positives as no translocations other than expected for cell lines were detected. Bone marrows of 36 patients suspended in lysis buffer were taken for routine genetic diagnosis (Karyotyping, FISH and or RT-PCR) and TLA. Sample analysis was performed randomized and blinded. TLA outcome was then compared with results from routine genetic testing.

Results: From a total of 36 patients three samples did not meet the required sample quality for further analysis. In the remaining 33 patients our TLA multiplex panel confirmed the presence of translocations on 16 samples. This includes a cryptic translocation involving the ETV6-RUNX1 fusion gene, t(12;21)(p13;q22) in five pediatric AML samples, not detected with karyotyping but RT-PCR, confirming the TLA findings. In fifteen samples no translocation was detected, yet RT-PCR, confirming the TLA findings. Three translocations were missed due to insufficient sequence reads on the partner chromosome. In addition, in one sample one translocation partner was also missed, located in the telomeric region of the chromosome and therefore resulting to nonspecific mapping of the sequence reads. An additional finding, involving a three way translocation t(9;22;11), missed by cytogenetics was detected by our panel. Two new findings have yet to be confirmed with FISH.

Summary/Conclusions: Our TLA panel showed concordant results for 29 out of the 33 successful sequenced samples. No false positives were found, while an additional translocation was detected. Our panel is able to detect (cryptic) translocations without prior knowledge of the fusion partner. Therefore, the TLA multiplex panel is suited as a first tier screening tool in acute leukemia. A prospective study, comparing the diagnostic yield of the TLA panel with current tests, can establish whether the TLA panel is applicable as a routine procedure.

E898
ALTERATIONS IN NECROPTOSIS PATHWAY AFFECT PROGNOSIS OF PATIENTS WITH ACUTE MYELOID LEUKEMIA
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Background: Necroptosis is a type of necrotic cell death involving several genes transcription and activation of molecular mechanisms as death receptors, interferon, toll-like receptors, intracellular RNA and DNA sensors. The process is leading by the family of receptor-interacting protein kinase (RIPK3, RIPK2, RIPK1) and the MLKL substrate. Losses of RIPK3 or MLKL, as well as deficiency in apoptosis, could allow tumor cells to escape the immune-mediated cells death (ICD).

Aims: We want to investigate the role of necroptosis deficiency in correlation with chemotherapy resistance and its impact as prognostic factor in AML.

Methods: We performed SNP Arrays (Cytoscan HD and SNP 6.0, Affymetrix) on a cohort of 300 non-M3 AML patients at diagnosis and we analyzed the Overall Survival (OS) of our patients with deficiency on necroptosis pathways. Survival was analyzed with Kaplan-Mayer method and Log-Rank test. We further analyze the relevance of different prognostic factors by the use of COX-Hazard Ratio statistical analysis.

Results: We found that 18 patients presented a loss of RIPK1 or MLKL (nobody presented losses in RIPK3/RIPK2) and 13/18 patients were older than 65 years old. The Overall Survival (OS) of patients with alterations in these genes is significantly lower than control group, with a median OS of 3 vs 6 months respectively (p<0.001). With Fisher Exact Test we further demonstrate that copy number loss of RIPK1 or MLKL are associate to loss of TP53 or FANCA genes, complex karyotype and advanced age. COX-Hazard Ratio model with RIPK1 or MLKL loss, BRCA1 loss, TP53 mutation, FANCA loss, secondary disease and diagnosis karyotype considered as categorical variable show that necroptosis deficiency (HR 1.98, CI 95% 1.04-3.78) TP53 mutation, and secondary AML are independent negative prognostic factors in an optimal model.

Summary/Conclusions: Our study shows that losses in necroptosis pathways are an uncommon alteration in AML, prevalent in old population. Moreover, we hypothesize that the loss of genes involved in necroptosis could be a real mechanism of tumor immune-escape and could be a rational to select patients that high probability to be resistant at chemotherapy promoting ICD mechanism.

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E999

NGS ANALYSIS AND IMPACT OF VARIANT ALLELIC FREQUENCY AT RELAPSE AND REFRACTORY STATUS IN AML PATIENTS

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Background: A high number of patients with acute myeloid leukemia (AML) present resistance at treatment, which is associated with clonal persistence or evolution. The generation of high-depth sequencing data allowed to quantify variant allelic frequencies (VAF) and permitting estimation of the size of tumor clonal populations in each AML sample, and to perform an estimation of clonal evolution at relapse or refractory case according at diagnostic.

Aims: To evaluate the predictive impact of the fluctuation Variant Allelic Frequency in resistance to treatment cases in AML.

Results: The variable frequency signalling pathway (EPOR, MLL, TET2, IDH1 and IDH2; p=0.043). Mutations in TET2, U2AF1 or SF3A1 showed VAF trend decreases. No correlation was found between VAF and% blasts, nor did VAF fluctuation with blasts fluctuation.

Summary/Conclusions: In conclusion, we provide preclinical evidence that combination of a TKI, especially midostaurin, with a MEKi, such as trametinib, is a rational and efficacious treatment regimen for AML. As trametinib has previously shown good results when combined with pazopanib in clinical trials for other kind of tumors, we expect similar results in AML.

This work was supported by the grant: PI13/02387.

E990

PRECLINICAL EVIDENCE THAT TRAMETINIB ENHANCES THE RESPONSE TO TYROSINE KINASE INHIBITORS IN ACUTE MYELOID LEUKEMIA

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Background: Acute Myeloid Leukemia (AML) is the most common type of acute leukemia in adults and the second in children in whom overall survival is less than 35% and 60% respectively. Activating mutations of FLT3 are now recognized as the most common molecular abnormality in this disease, and the poor prognosis of patients harboring these mutations renders FLT3 an obvious therapeutic target. Although different tyrosine kinase inhibitors (TKI) have been used for this purpose, their ability to extend progression-free and overall survival is limited by drug resistance. This strategy could be improved by rationally combining TKIs with other agents. In this work, we have explored bone marrow samples from a FLT3-AML patient before and after TKI treatment by phospho-proteomics and observed enhanced activity of Ras-Raf-MEK-ERK1/2 pathway as a possible mechanism for TKI resistance.

Aims: To validate the role of ERK1/2 during TKI resistance in vitro and ex vivo and to search suitable combinations that allow overcome/avoid resistances in preclinical models of the disease.

Methods: Resistance mechanisms were studied in vitro in MOLM13 (FLT3WT/ITD) after generating resistance by two different methods: by subculturing with increasing doses of sorafenib or by treating them with high doses of sorafenib, and recollecting alive proliferative (resistant) cells after CFDA and Annexin labeling. Phosphoproteomic analyses were carried out by LC-MSMS after IMAC enrichment or by western blot techniques. Drug sensitivity assays with trametinib (MEK inhibitor) and three TKIs (sorafenib, pazopanib, midostaurin) were read after 48 hours or 72 hours of treatment in vitro or ex vivo respectively. The efficacy of the combinational treatments was characterized by the cell viability assay using WST8, and analyzed with Graphpad Prism software. Synergy effects were measured with Calculusy software.

Results: As it is presented in figure 1, ERK1/2 pathway was more activated after TKI treatment in the FLT3-AML patient during sorafenib-resistance development. The same fact was confirmed in MOLM13 sorafenib-resistant culture and in living proliferative cells recollected after sorafenib treatment. Different doses of trametinib, sorafenib, pazopanib and midostaurin in monotherapy were tested in MOLM13 cell line determining their IC50 values. Synergy effects of combining trametinib with the three TKIs were analyzed with Calculusy software.

Figure 1.
E901
IDENTIFICATION OF NOVEL THERAPEUTIC DRUGS IN DISTINCT PEDIATRIC AML SUBTYPES BY TARGETING EPIGENETIC REGULATORS
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Background: Treatment protocols for pediatric acute myeloid leukemia (AML) are chemotherapy-based, including high-dose cytarabine. While >90% of patients reach clinical remission, there is still a high relapse rate of ~30%, with overall survival rates of 60-70%. Therefore, better risk-classification at diagnosis and alternative treatment strategies are warranted. There is increasing evidence that epigenetic deregulation is involved in the initiation and progression of cancers, including AML. Epigenetic processes are required for hematopoiesis and epigenetic regulators are frequently translocated (MLL) or mutated (EZH2) in AML. Following this, deregulated epigenetic pathways could be used for targeted therapy and provide an alternative approach to improve pediatric AML therapy.

Aims: To identify new therapeutic drugs in pediatric AML by using an 80-compound screen containing inhibitors of epigenetic regulators, including histone writers (which deposit post-translational modifications (PTMs) on histones), readers (binding of PTMs) and erasers (removal of PTMs).

Methods: Cell lines used in this study are THP-1 (t(9;11)), Kasumi-1 (t(8;21)) and CMK (Down’s syndrome with GATA1 mutation), reflecting distinct pediatric AML entities and a differential response to treatment with cytarabine. Cells were treated for 72hrs followed by analysis of cell viability and apoptosis based on Hoechst, Draq7 and Calcein Green staining. The effect of three candidate compounds were further investigated in triplicates at several concentrations for their effect on cell viability (Annexin V/FITC staining), cell cycle, morphology, and colony formation. Dose-response curves showed differential cytotoxicity of the compounds and suggested LMK235 as most effective. Cell proliferation was inhibited by LMK235 at an IC50 of 0.1µM, 0.13µM and 0.425µM in Kasumi-1, CMK and THP-1, respectively. While inhibition by LMK235 resulted in an immediate response of apoptosis, Bromosporine-treated cells retained in G1 phase of cell cycle, and, interestingly, CMK cells showed an increase of cells in S-phase and G2/M. Among the differential effects of the compounds in the cell lines, we also observed differences in sensitivity. In line with previous studies, THP-1 cells were more resistant, illustrated by a 10-fold increase in concentration required for NSC3852-induced apoptosis. Interestingly, upon IFNα treatment, Kasumi-1 and CMK cells showed a similar response, while Kasumi-1 cells were significantly more sensitive to NSC3852-induced effects. These data are currently validated in pediatric AML patient cells.

Summary/Conclusions: Treatment of three distinct pediatric AML cell lines with the epigenetic compounds LMK235, NSC3852 and Bromosporine resulted in cell line-specific effects, regarding compound sensitivity, and compound specific effects, including cell cycle regulation and induction of apoptosis. Our data suggests a potential role for epigenetic compounds, with specificity for molecular subtypes, in the treatment of clinically and biologically distinct pediatric AML subtypes.

E902
ALVOCIDIB SYNERGIZES WITH CYTARABINE AND DAUNORUBICIN (7+3) IN PRECLINICAL MODELS OF ACUTE MYELOID LEUKEMIA
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Background: Interferon alpha (IFNa) monotherapy is effective in selected myeloid neoplasias and is proposed to act through mechanisms that may be additive to the action of cytarabine (VPA), a histone deacetylase (HDAC) class I and IIA inhibitor with effect in approximately 20% of acute myeloid leukemia (AML) patients. Normal myeloid drugs are found to have direct anti-cancer effects targeting apoptosis, differentiation and proliferation, as well as indirect effects targeting the immune system.

Aims: As several IFNa formulations are commercially available, we wished to explore the differences between two such drugs, recombinant IFNα-2b and human IFNα-Le, in relevance to AML treatment.

Methods: Flow cytometry and Hoechst staining was used to investigate apoptotic potential of the IFNα therapeutics, whilst phospho-flow cytometry and difference gel electrophoresis in combination with mass spectrometry unraveled IFNα signaling pathways. For in vivo effectivity analyses two orthotopic rodent models were implanted with leukemia cells and treated with VPA, IFNα-Le or both drugs.

Results: To investigate the anti-leukemic effects of IFNa we combined the two therapeutics with VPA in vitro using the human MOLM-13 cell line (wild type for FLT3 ITD and TP53). Results showed that IFNα-Le was more efficient compared to IFNα-2b in inducing apoptosis, whilst both were synergistic in combination with VPA. Investigating IFNα signaling pathways using phospho-specific flow cytometry we found IFNα-Le to have an identical stimulation profile in MOLM-13 cells, except from p-STAT5 Y691 that was higher expressed by IFNα-2b. The phospho-proteome was further explored using difference gel electrophoresis (DIGE) and mass spectrometric analyses to find a potential explanation to the difference in apoptosis-inducing effects between the two drugs. Here we found protein folding (LCP1, HSPA8, TCP1, CCT6A), cell stress (AKR1B1, HPVOA1B) and cell death (PKM2, PARK7, HSPB1, HSPA5, ANX5, PRDX2) to be differently regulated between IFNα-2b and IFNα-Le, and identified the presence of cell-line-dependent effect on protein expression by IFNα-2b and IFNα-Le. Further, we investigated the potential synergistic anti-leukemic effects of VPA and IFNα-Le in vivo using a MOLM-13Luc-immunodeficient NOD/Scid IL2 g-/- orthotopic xenograft mouse model, and the
immunocompetent brown Norwegian myeloid leukemia (BNML) syngeneic rat model. VPA mono-treatment increased survival from a median of 34 days to 38 days in the MOLM-13BH4 mouse model, and from 21 days to 50 days in the BNML rat model. Additionally, the IFNα-Le (0.8x10⁶ IU/kg) and VPA (400mg/kg) combination treatment indicated a tendency to increased survival in the BNML model. However, IFNα-Le monotherapy (1x10⁹ IU/kg) decreased survival in the MOLM-13BH4 model.

Summary/Conclusions: Our data indicate that Kevetrin exposure induces cell growth arrest, a great drop of mitochondrial membrane potential and a remarkable increment of Caspase-3 cleaved form, features that contribute to apoptotic cell death in the two cell lines. Cellular changes can be associated with a dose and time-dependent effect in the TPS3 mutated cell line (KASUMI-1) but not in the wild type one (MOLM-13), in which we can observe an activity only after 48 h at the higher concentration. Regarding molecular alterations in KASUMI-1 we found a great p53 down-regulation, probably due to Hsp90 reduction, resulting in a less marked formation of the Hsp90-p53 oncogenic complex. We also found a down-regulated p53 active form (Ser15), a reduced expression of p53 targets, p21 and PUMA, and a down-regulation of SIRT-3, that cannot exert its inhibitory activity on p53. The MOLM-13 cell line showed a great p53 reduction, probably related to SIRT-3 up-regulation and Hsp90 down-regulation. Regarding p53 active form, we noticed slight variations in protein expression, suggesting a physiological response of the protein to cellular damage. In accordance with p53 activity, we observed a great reduction of p21, probably associated with a drug resistance mechanism; in contrast, PUMA protein was highly down-regulated, suggesting a p53-independent mechanism of action or a feedback regulation of the apoptotic process, after Caspase-3 activation (Figure 1). In order to better understand drug’s mechanism of action we are performing gene expression profiling after 48h of treatment with Kevetrin 80μg/ml.

E904
KEVETRIN: PRECLINICAL STUDY OF A NEW COMPOUND IN ACUTE MYELOID LEUKEMIA
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Background: Acute Myeloid Leukemia (AML) is a heterogeneous disorder defined by clonal expansion of immature myeloid cells that infiltrate bone marrow and other tissues. AML therapeutic strategies remain unchanged since 1970 and the majority of patients often eventually relapse and die due to disease progression. Tumor protein p53 transcription factor is a key regulator of several cellular pathways, such as cell cycle, apoptosis and angio genesis. It is mutated in 8-14% of AML cases and its mutations are commonly associated with a complex karyotype. Kevetrin is a new molecule compound, proposed by Cellceutix, with the ability to target both wild type and mutant p53 tumors.

Aims: The aim of this project is to explore cellular and molecular alterations induced by Kevetrin, focusing on its role in the p53 pathway.

Methods: Kevetrin was kindly provided by Cellceutix, dissolved and stored at 4°C in sterile water in a 600 μg/ml stock solution, and diluted in medium immediately before use [concentration range in use 15-60μg/ml]. Cell lines, MOLM-13 and KASUMI-1 were cultured in RPMI 1640 supplemented with 20% heat inactivated fetal bovine serum, 2mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. After 24 and 48 h of treatment MTS, Annexin-V, TUNEL, JC-1 and Active Caspase-3 assays were performed according to manufacturer's instructions. Proteins were separated by polyacrylamide gel electrophoresis and transferred to 0.2 μm polyvinylidene fluoride membranes. Quantitative analysis was performed with Quantity One software. Statistical analysis was carried out using the paired and unpaired two-tailed Student’s t tests. p values <0.05 were considered as significant.

Results: Our data indicate that Kevetrin exposure induces cell growth arrest, a great drop of mitochondrial membrane potential and a remarkable increment of Caspase-3 cleaved form, features that contribute to apoptotic cell death in the two cell lines. Cellular changes can be associated with a dose and time-dependent effect in the TPS3 mutated cell line (KASUMI-1) but not in the wild type one (MOLM-13), in which we can observe an activity only after 48 h at the higher concentration. Regarding molecular alterations in KASUMI-1 we found a great p53 down-regulation, probably due to Hsp90 reduction, resulting in a less marked formation of the Hsp90-p53 oncogenic complex. We also found a
stem cells contributing to clonal haematopoiesis (Askush et al, Nature 2014; Genovese et al, NEJM 2014); this led us to study the distribution of RUNX1 gene variants in an additional 119 AML diagnostic samples. 34 patients (21%) harboured RUNX1 mutation, of which 5 had RUNX1_L56S that were often associated with D-C mutations (4 of 5 cases). Finally, we evaluated kinetics of D-C in 40 MDS cases of which 34 had chronic MDS and 6 had secondary AML (sAML). No significant difference was observed in the number of patients with persistent D-C mutation in the 2 subgroups (chronic MDS: 16 of 19 (84.2%); sAML: 5 of 5 (100%); P-value: 1.000).

Table 1.

Summary/Conclusions: Clearing of ‘Driver-COSMIC only’ mutations while RUNX1_L56S persists is unlikely to contribute towards disease progression in AML.

Acute myeloid leukemia - Clinical

E906

PROGNOSTIC SIGNIFICANCE OF FLT3 STATUS, CYTOGENETIC, ECOG AND 50% BLAST DECREASE IN PRIMARY REFRACTORY OR EARLY RELAPSED AML PATIENTS BEFORE SALVAGE THERAPY

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Background: Prognosis of relapsed/refractory acute myeloid leukemia (R/R AML) is unfavorable with a long term overall survival around 10%. Thus, management of R/R AML represents one of the most difficult challenges. Because allogeneic-Hematopoietic Stem Cell Transplantation (allo-HSCT) is considered as the best treatment for this category of patients, to determine which patient will benefit from this cumbersome strategy is a crucial issue. A better understanding of the mutational status, cytogenetic, histological and clinical findings of early R/R AML patients and their outcomes could help treatment decisions, particularly for those who allo-HSCT is considered as the best therapeutic option.

Aims: The objective of this study is to determine prognostic factors and develop a prognostic score using usual mutational status, cytogenetic, histological and simple clinical variables in R/R AML patients before salvage treatments.

Methods: In this retrospective study in two hematological departments (Hospices Civils de Lyon and CHU of Toulouse), we evaluated clinical, biological, histological, cytogenetic and current mutational status of early R/R non APL AML patient between age from 18 to 70 years. Univariate and multivariate analysis were performed and we developed a prognostic score based on the independent prognostic parameters from Cox model.

Results: From January 2009 to May 2016, 58 patients presenting early relapse and primary refractory AML were analyzed. Overall Survival (OS) and Progression Free Survival (PFS) median were 9 and 2 months respectively. In univariate analysis, cytogenetic findings (unfavorable groups), unfavorable ECOG (>2), FLT3 positive status and <50% blast decrease (between induction and R/R assessment) independently predicted poor OS and were identified as significant prognostic parameters of OS (p=.037, p=.0084, p=.0452, p=.0071 respectively). In multivariate analysis, these last four criteria confirmed their worst prognostic impacts (p=.015, p=.017, p=.026, p=.015 respectively) and were used to create a five groups prognostic score. Better OS were statistically observed for patient with score 0 or 1 compared to 2, 3 or 4 (2-years OS 48% and Not Reached respectively, p=.0104) using a log-rank regression. When data were censored to allo-HSCT, the scoring system revealed a relevant difference with favorable OS for those with a score 0-1 compared to score 2-3-4 (2-years OS 64% and Not Reached respectively, p=.001) (Figure 3).

Figure 1.

Summary/Conclusions: Our prognostic score based on simple and usual data: FLT3 status, cytogenetic, ECOG and percentage blast decrease found distinct groups with statistically different outcomes. Basically, the higher is the score, the worst is the OS. This new score is a valuable, simple and useful score for the therapeutic salvage management of AML patients presenting early relapse and primary refractory.
PRELIMINARY RESULTS FROM A PHASE 1 STUDY EXAMINING THE NOVEL BCL-2 INHIBITOR S55746/BCL201 AS SINGLE AGENT IN PATIENTS WITH ACUTE MYELOID LEUKEMIA OR HIGH RISK MYELODYSPLASTIC SYNDROME

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Background: Novel and effective therapeutic options for patients (pts) with advanced acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) are limited. Targeting the pro-survival molecule BCL-2 is clinically efficacious in various hematological malignancies. S55746/BCL201 is a novel, selective and potent inhibitor of BCL-2, with demonstrated antileukemic activity in preclinical models.

Aims: To evaluate the safety, recommended phase 2 dose (RP2D), pharmacokinetic (PK), pharmacodynamic (PD) and preliminary activity of S55746/BCL201 in patients with AML (relapsed/refractory (R/R) or ≥65 years unfit for intensive chemotherapy (CI)), or MDS failing prior therapies.

Methods: A phase 1 study (EUDRACT 2014-002559-24, NCT02920541) is underway to investigate S55746/BCL201 as a single agent in 5 European and Australian centers. S55746/BCL201 was initially administered in fasting conditions, once daily (21-day cycles), until disease progression, unacceptable toxicity, or investigator’s or patient’s decision. Pts giving informed consent received S55746/BCL201 at escalating dose levels according to a modified continual reassessment method for dose allocation.

Results: As of 23 February 2017, 34 pts have received S55746/BCL201 at doses ranging from 100 to 1200mg/day (median time on treatment: 43 days, range 1 to >374), 28 pts were R/R AML, 2 pts were elderly AML unfit for CI, and 4 pts had MDS failing prior therapies. Median age was 70 years (range 19-80), median number of prior therapies 2 (range 0-6), ECOG ≤2, and median WBC 4×10^9/L (range 0.9-53). Among 79 cycles of treatment, 53 pts (100%) were evaluable for safety, 31 pts (69%) evaluable for PD and 14 pts (32%) evaluable for drug concentration.

Summary/Conclusions: Initial findings suggest that S55746/BCL201 has acceptable tolerability and clinical activity in advanced AML and MDS. Based on non-compartmental pharmacokinetic food interaction results from another study, demonstrating that S55746/BCL201 Cmax and AUC increased about 6-fold with food, dose escalation has started in patients with drug intake during a meal.
Aims: The authors evaluated patients with AML to determine expression levels of checkpoint molecules (PD-1, PD-L1, and PD-L2) according to diagnosis and treatments (chemotherapy [CTx] and SCT). The purpose of this study was to identify optimal candidates for checkpoint blockade therapy for AML.

Methods: Bone marrow (BM) samples were obtained from 195 AML patients in different stages of the disease. Samples were stratified by time of diagnosis (n=69) and treatment response (complete remission [CR] after CTx, n=30; persistence after CTx, n=29; relapse after CTx, n=7; normocellular marrow with trilineage regeneration [NMTR] after SCT, n=19; persistence after SCT, n=18; and relapse after SCT, n=23). BM samples also were collected from 23 patients with no evidence of hematologic malignancies (control group). Flow cytometric analysis of PD-1 expression on T cells and PD-L1/PD-L2 expression on leukemic cells was performed by means of a FACSCanto II system (Becton-Dickinson, Sunnyvale, CA, USA).

Results: There were no differences in levels of PD-1 expression on CD8+ and CD4+ T cells at time of AML diagnosis, compared with controls. However, PD-1 expression levels on CD4+ T cells were significantly correlated with time since diagnosis. For patients at time of diagnosis, PD-1 expression on CD8+ and CD8+ T cells were significantly different compared with patients who experienced relapse after SCT (P<.0001), persistence after SCT (P=.0001), and NMTR after SCT (P=.0001). In contrast, no difference in PD-1 expression was observed between patients at time of diagnosis and patients after CTx (Figure 1). For CD4+ T cells, a significant difference was found between SCT and CTx groups, and PD-1 expression levels of groups that experienced relapse (P<.0001) or persistence (P<.0001) after SCT were significantly higher than those of patients in the CTx groups. PD-L1 and PD-L2 expression on leukemic cells at time of diagnosis was higher in secondary AML transformed from myelodysplastic syndrome than in de novo AML (P=.0001 and P=.039). Although PD-L1 and PD-L2 expression levels for patients at time of AML diagnosis did not differ from groups that experienced relapse or persistence after SCT, PD-L1 and PD-L2 levels for diagnosed patients did differ from those of patients who experienced persistence after CTx (P=.038 and P=.023).

Figure 1.

Summary/Conclusions: Our study suggests that the PD-1/PD-L1 pathway may constitute an immune-escape mechanism in AML. PD-1 expression in CD4+ T cells increased with time since diagnosis. Patients who underwent SCT exhibited overexpression of PD-1, which suggests that SCT and/or chronic stimulation of leukemic cells might induce more PD-1 expression by T cells. Blockade of the PD-1 immune checkpoint may represent an immunotherapeutic strategy for patients with AML relapse or AML persistence after SCT.

E910

ACUTE LEUKEMIA IN HIV PATIENTS: EPIDEMIOLOGY, THERAPEUTIC STRATEGY AND PROGNOSIS

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Background: Data on HIV patients with acute leukemia (AL, acute myeloid leukemia (AML) or non Burkitt acute lymphoid leukemia (ALL)) are very poor especially on their outcome. Treatment of acute leukemia usually depends of patient-related prognostics factors and disease related prognostic factors. Because HIV patients are considered frail, they are always excluded of therapeutic protocols. There are no guidelines for their treatments.

Aims: Our aim was to precise the epidemiology, the best therapeutic strategies as well as patient’s prognostics, and to compare their outcome to those of seronegative patients with AL.

Methods: We conduct a retrospective national multicentric study. HIV positive patients with a diagnosis of AML or non Burkitt-ALL between January 2000 and February 2016 were included. We compared HIV patients’ outcome to those of seronegative patients with AL after a propensity score matching.

Results: 47 HIV patients with a diagnosis of AL (42 AML and 5 ALL) were included. AL incidence in HIV patients (HIVP) is not different than in general population but AL occurred earlier (49.29 years [44.21 ; 57.47]) and secondary AL are more frequent (42.55%). With a global and multidisciplinary approach these patients can be treated with intensive chemotherapy resulting on good efficiency (complete remission [CR]=84.38%) and tolerance. Based on a multivariable model, only absence of CR was associated with hazard of death (p=0.01). 8 patients (17.02% ; 7 AML and 1 ALL) received a hematopoietic stem cell transplantation. HIVP with AL 2 years overall survival (OS) was 29% CI95% [15 ; 54] for AML and 40% CI95% [14 ; 100] for ALL. There was no difference in OS between our HIVP and seronegative controls with AL after propensity score matching (HR=1.347 [0.6486-2.796]; p=0,42).

Table 1.

Summary/Conclusions: Our study shows that HIV status has no prognostic impact on AL patient’s outcome. HIV patient with acute leukemia should thus be included in clinical trials to improve and standardize their therapeutic management.

E911

TEN-DAY DECITABINE AS INDUCTION THERAPY FOR OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA FIT FOR INTENSIVE CHEMOTHERAPY

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Aims: Our aim was to precise the epidemiology, the best therapeutic strategies as well as patient’s prognostics, and to compare their outcome to those of seronegative patients with AL.

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Results: 47 HIV patients with a diagnosis of AL (42 AML and 5 ALL) were included. AL incidence in HIV patients (HIVP) is not different than in general population but AL occurred earlier (49.29 years [44.21 ; 57.47]) and secondary AL are more frequent (42.55%). With a global and multidisciplinary approach these patients can be treated with intensive chemotherapy resulting on good efficiency (complete remission [CR]=84.38%) and tolerance. Based on a multivariable model, only absence of CR was associated with hazard of death (p=0.01). 8 patients (17.02% ; 7 AML and 1 ALL) received a hematopoietic stem cell transplantation. HIVP with AL 2 years overall survival (OS) was 29% CI95% [15 ; 54] for AML and 40% CI95% [14 ; 100] for ALL. There was no difference in OS between our HIVP and seronegative controls with AL after propensity score matching (HR=1.347 [0.6486-2.796]; p=0,42).

Table 1.

Summary/Conclusions: Our study shows that HIV status has no prognostic impact on AL patient’s outcome. HIV patient with acute leukemia should thus be included in clinical trials to improve and standardize their therapeutic management.
Leukemia Net risk stratification, 9 had favorable risk (3 with DNMT3A and 2 with low allelic ratio (<0.5 of FLT3-ITD) and 3 had adverse risk. No RLT was observed with the 1st and 2nd dose levels. The most frequently reported adverse events (regardless of attribution), were febrile neutropenia, diarrhea, nausea and vomiting, dyspnea, hypotension, and hypoxia. Three pts are no longer on study: 1 (dose level 0) due to inability to swallow indoximod after hypoxic respiratory failure during induction, 1 (dose level 1) withdrew consent for personal reasons after only 2 doses of indoximod, and 1 (dose level 2) was taken off due to eligibility. The remaining 9 pts are still on study; 3 pts in dose level 2 are currently receiving induction and are not evaluable. Five of 6 (83%) evaluable pts in dose levels 0 and 1 achieved complete remission (CR) after induction. All 5 pts demonstrated no evidence of MRD at levels <0.02% (MRD-neg) post-induction and remained MRD-neg post cycle 1 of HiDAC. One pt in dose level 1 with favorable risk (normal karyotype, mutations in DNMT3A/NPM1/NRAS) had primary refractory disease. The pt who was unable to swallow indoximod had a favorable risk (normal karyotype, mutations in DNMT3A/NPM1) and achieved morphologic CR but had MRD at the end of induction, and ultimately relapsed after 2 cycles of HiDAC consolidation.

Summary/Conclusions: Indoximod does not appear to add significant toxicity to standard remission induction and consolidation in pts with newly diagnosed AML. Initial data suggest a high rate of MRD-neg after one cycle of induction chemotherapy.

E913

PHASE III STUDY OF MEK INHIBITOR (MEK-162; BINMETINIB) IN PATIENTS WITH RELAPSED AND/OR REFRACTORY MYELOID MALIGNANCIES

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Background: Activation of the mitogen-activated protein kinase (MAPK) signaling (RAF/RAF/MEK/ERK pathway) promotes growth and inhibits apoptosis of hematopoietic cells. Inhibition of MEK/MEK/MEK pathway has shown antiproliferative effects in acute myeloid leukemia (AML) cell lines and AML blasts. MEK-162 is an oral, potent, selective allosteric, ATP non-competitive inhibitor of MEK1 and 2.

Aims: To study the efficacy and safety of MEK-162 in patients with advanced myeloid malignancies.

Methods: Patients with relapsed/refractory AML, not candidates for intensive chemotherapy, and patients with high risk myelodysplastic syndrome (MDS) who were resistant/intolerant to standard treatment including stem cell transplant were treated with MEK-162 twice daily every 28 days. Patients in the expansion phase had to be RAS mutated. The primary endpoint was overall response rate (ORR=CR + CRi) after 1 cycle of therapy. Survival was estimated using the Kaplan-Meier method. Safety analysis included all patients who had received at least 1 dose of MEK-162. MEK-162 dose escalation followed a 3+3 design; phase 2 had built in futility/toxicity boundaries. 45mg twice daily is the final dose level for expansion phase.

Results: Sixteen patients were treated (escalation=7; expansion=9): 14 AML and 2 MDS. Median age was 62 years (31-85); 56% were male; 94% had a performance status of 1-2. Median number of prior therapies was 4 (1-6). 3/16 (19%) patients had complex karyotype. 11/69 (16%) patients were RAS mutated. Among 16 patients completed a minimum of 1 cycle of MEK-162 therapy and were evaluable for response (3 at 30mg and 7 at 45mg dose). ORR was 10% (CRI in 1/10 patients). Median number of cycles administered were 2 (1-4). Median duration on therapy was 1.1 months (0.1-3.4). Median overall survival is 3 months (0.3-7.6). Common G3/4 toxicity included neutropenia (56%), fatigue (13%), nausea/vomiting (13%) and electrolyte abnormalities (19%). No dose limiting toxicity was reported.

Summary/Conclusions: MEK-162 shows a tolerable safety profile with an ORR of 10%. The study is currently on-going. Additional studies involving combination of MEK-162 with RAF and PI3 kinase inhibitors are ongoing.

E914

HAPLOIDENTICAL TRANSPLANTATION IS SAFE AND EFFECTIVE FOR OLDER PATIENTS WITH AML/MDS

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Background: Acute myeloid leukemia (AML) is more common in the older population. Haploidentical stem cell transplantation (haploSCT) is a potentially curative...
ative treatment option for patients with AML and allows transplantation for patients without an HLA matched donor. Recently, the use of post-transplant cyclophosphamide-based (PTCy) GVHD prophylaxis has improved outcomes of haploSCT, however, outcomes of haploSCT in older patients remain unclear.

**Aims:** Here we evaluated outcomes of older patients with AML/MDS who underwent haploSCT.

**Methods:** We retrospectively analyzed outcomes of all 43 patients ≥55 years with AML/MDS who underwent a haploSCT at our institution after year 2009. All patients were treated with fludarabine-melphalan (FM)-based conditioning regimen (melphalan 100 or 140mg/m²) plus thiotepa 5mg/kg or 2GyTBI. Characteristics of these patients are presented in Table 1.

**Results:** Median age was 61 years (range 55-69), 22 patients (51%) were in CR1/2, 16 patients (37%) had poor-risk cytogenetics, and median HCT-CI was 2 (range 0-11). Reduced melphalan regimen (100mg/m²) was used in 29 pts (67%). Donors were children in 35 (81%) or siblings 10 (19%) patients. Median follow-up was 19 months (range 6-49). One patient died prior to engraftment. Forty-two patients engrafted the donor cells (100%). Median time to neutrophil and platelet engraftment was 19 (13-28) and 28 (15-117) days. Day 30 chimerism was 100% donor in 38 patients (88%). The cumulative incidence of NRM was 21%, 30% and 5% while CI of cGVHD at 2 years post-transplant was only 9%. The 2-year rate was 24%. Cumulative non-relapse mortality (NRM) was 21%, 30% and 34% at day 100, 1 year, and 2 years post-transplant. Patients in CR1/2 had 2-year NRM and relapse rate of 23% and 14%, and OS was 61%. The 2-year OS for patients in CR1/2 with intermediate/favorable-risk cytogenetics was 73%. In multivariate analysis, favorable predictors for OS were CR1/2 (HR:0.4, p=0.05), good/intermediate cytogenetics (HR:0.2, p=0.01), and donor age greater than 40 (Figure 1).

**Figure 1.**

**Table 1.**

Summary/Conclusions: HaploSCT with PTCy-based GVHD prophylaxis is safe and effective for older AML/MDS patients. Lack of an HLA matched donor is not a contraindication to proceeding to a haploidentical transplant in older AML/MDS patients. In addition to remission status and cytogenetics, we found that younger donor age was significantly associated with improved survival in older AML/MDS patients undergoing haploidentical transplantation.

**E915**

**OPTIMIZATION OF MINIMAL RESIDUAL DISEASE EVALUATION IN ACUTE MYELOID LEUKEMIA TO DRIVE POST-INDUCTION THERAPY**

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**Background:** Among Acute Myeloid Leukemia (AML) patients achieving hematological complete remission (CR) the persistence of detectable disease assessed with highly sensitive techniques as Multicolor-Flow Cytometry (MFC) or PCR-based molecular analysis retains a negative prognostic value. However, a consensus on the most informative time-points (TP) and sensitivity cut-offs for MRD assessment has not been reached.

**Aims:** The aim of the present study was the evaluation of the prognostic impact of MFC and molecular MRD assessment by identifying TP, MFC positivity cut-off values and molecular MRD markers with the highest prognostic impact.

**Methods:** One hundred and ten consecutive AML patients treated in our center between 2004 and 2014 were retrospectively analyzed. As previously described, all patients had a fludarabine-containing induction. Median age was 47 years (range 18-65). Median follow up was 59 months. Three different MRD TP have been considered: TP1, after induction I; TP2, after induction II; TP3, after consolidation therapy for patients who did not undergo hematopoietic stem cells transplantation (HSCT). For patients who underwent HSCT, TP3 coincided with pre-transplant MRC evaluation. MFC-MRD evaluation had been performed through 4-colour MFC analysis (and 8-colour from 2013). To define MFC-MRD positivity two cut-offs were considered: a threshold of 2.5×10−4 residual leukemic cells (>0.025%) or a threshold of 1×10−3 residual leukemic cells (>0.1%). For patients carrying NPM1-gene mutation NPM1 expression levels at TP1, TP2, TP3 (NPM-MRD) were analyzed. A reduction >3.5 log of NPM1 transcript at TP1 was considered optimal as per our published experience. For patients presenting WT1 over-expression at diagnosis WT1-MRD was evaluated at TP1, considering WT1 negativity with a cut-off of WT1 copies/10^4 ABL lower than 250.

**Results:** CR rate after induction I and II was 82.7 and 85.5%, respectively. The percentage of MFC-MRD negativity at TP1 was 0.025% increased from TP1 to TP2. However, the probability of achieving MFC-MRD negativity at TP2 was only influenced by ELN risk group (p<0.05). In the whole cohort, 2 years OS was 60.2% (median not reached). Multivariate Cox-Proportional Hazard model showed that MFC-MRD >0.1% at TP2 was the strongest predictor of higher risk of death, whereas MFC-MRD <0.025 at TP1 was the strongest predictor of long survival (Figure 1). To define the MFC-MRD positivity two cut-offs were considered: a threshold of 2.5×10−4 residual leukemic cells (>0.025%) or a threshold of 1×10−3 residual leukemic cells (>0.1%). For patients carrying NPM1-gene mutation NPM1 expression levels at TP1, TP2, TP3 (NPM-MRD) were analyzed. A reduction >3.5 log of NPM1 transcript at TP1 was considered optimal as per our published experience. For patients presenting WT1 over-expression at diagnosis WT1-MRD was evaluated at TP1, considering WT1 negativity with a cut-off of WT1 copies/10^4 ABL lower than 250.

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**Figure 1.**

**Summary/Conclusions:** Our data show that MRD assessment at different time-point is a strong prognostic indicator and that different cut-offs at different time-points can give different and useful prognostic information that may drive post-induction therapy. MFC MRD evaluation at TP2 with 0.1% cut-off is the most useful for patients risk stratification. However, the evaluation of MFC-MRD at TP1 with 0.025% cut-off can early identify a group of patients with a significantly low risk of relapse. At the same TP, MFC and WT1 MRD integration allows a better risk stratification. MFC MRD is accurate also in NPM1-mut patients; however, in this cohort NPM1-based MRD evaluation is the most accurate predictor of prognosis.

**E916**

**THE NUMBER OF CD34+CD38+CD117+HLA-DR+CD13+CD33+ CELLS INDICATES POST-CHEMOTHERAPY NEUTROPHIL RECOVERY IN PATIENTS WITH ACUTE MYELOID LEUKEMIA**

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Background: Hematopoietic recovery is considered to be associated with the number of multipotent hematopoietic stem cells in the bone marrow, as observed in functional assays involving cell transplantation. However, there is little evidence related to hematopoietic recovery in non-transplantation settings, which is accomplished by endogenous hematopoietic cells. A recent study suggested that progenitors are the main contributors during this steady-state hematopoiesis, which differs from exogenous transplantation. And our previous data revealed that, CD34+CD38+CD117+HLA-DR+CD13+CD33+ cells (P cells), a kind of progenitor cell, is significantly decreased in patients with delayed neutrophil recovery after chemotherapy compared with that without delayed count recovery.

Aims: To further examine a potential impact of P cells percentage on hematopoietic recovery.

Methods: The data of 223 patients diagnosed with de novo AML was analyzed retrospectively. All these patients enrolled in our previously registered prospective randomized clinical trial AML 2010-01(201002024). We reviewed the data from bone marrow flowcytometry before the first and second course of consolidation therapy, in which the CD34+CD38+CD117+HLA-DR<CD13+CD33+ progenitor cell percentage in the bone marrow was analyzed. Plasma recovery time and time of neutropenia were counted for the evaluation of hematopoietic recovery ability after chemotherapy.

Results: We found that less P cell percentage was significantly associated with prolonged neutropenia recovery time after the first and second courses of consolidation chemotherapy (p=0.001; p=0.028, respectively). We also observed similar results regarding platelet recovery time after the first course of consolidation chemotherapy (p=0.001).

Univariate analysis showed that P cell percentage, rather than gender, age, WHO classification and cytogenetic subgroup, were associated with neutrophil recovery after chemotherapy. Multivariate analysis demonstrated that P cell percentage was an independent factor of delaying recovery of neutrophils, suggesting that P cells may be affecting neutrophil recovery capability for both first and second courses (p=0.015; p=0.036, respectively).

Summary/Conclusions: Our results indicate that CD34+CD38+CD117+HLA-DR<CD13+CD33+ cells before each course of chemotherapy is associated with chemotherapeutic hematopoietic reconstitution capacity independently. These findings may help better understand endogenous hematopoietic reconstitution and modify future chemotherapy regimens based on progenitor cell percentages.

E918 CYTOKINE RECEPTORS AND SOLUBLE ADHESION MOLECULE LEVELS ARE ASSOCIATED WITH PROGNOSIS OF NEWLY DIAGNOSED AML T. Kupsa1, P. Zák2, L. Jébavý1,2, J. M. Horacek1,2*
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Background: The outcomes of acute myeloid leukemia (AML) treatment are beleaguered by the high resistance of malignant clones to therapy. Cytokines and adhesion molecules have been studied as markers of immune system activation in many diseases including AML: Further knowledge gained from baseline cytokine levels assessment may help to improve treatment outcomes.

Aims: The aim of this study is to evaluate baseline levels of selected cytokines, cytokine receptors and adhesion molecules and their relationship with prognosis in newly diagnosed AML patients.

Methods: A total of 75 AML patients, age 52.9±13.0 years, median 58.5 years, 44 female, were studied in the period 2010-2015. Only patients with minimal follow-up of 1 year were included. All patients were induced with “3+7” induction chemotherapy consisting of Cytarabin 100mg/m² for day 7 consecutive days and Daunorubicin 90mg/m² for the first 3 days of therapy in younger patients. Since the beginning of 2015, the induction dose of Daunorubicin used has been 60mg/m² even in younger patients, according to recent evidence-based data modifications. Those who failed to achieve CR were given PAGL salvage chemotherapy followed by allogeneic stem cell transplantation in younger and fit patients. In CR, the patients were treated either with HIADAC consolidation and allogeneic stem cell transplantation or with their intensification therapy.

A total of 39 patients underwent allogeneic stem cell transplantation in our study. We evaluated serum levels of the following 29 analytes: interleukins (IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15), Erythropoietic Growth Factor, Granulocyte Macrophage Colony Stimulating Factor, Interferon-γ, Macrophage Inflammatory Protein-1α, Monocyte Chemoattractant Protein-1, Tumor Necrosis Factor-α (TNF-α), vascular endothelial growth factor, E-selectin (E-selectin), P-selectin (P-selectin), Intercellular Adhesion Molecule-1 (ICAM-1), Vascular Cell Adhesion Molecule-1 (VCAM-1), Matrix Metalloproteinase-9, soluble IL-2 receptor-alpha (sIL-2Ra) and soluble receptors for IL-6 (sIL-6R) and TNF-α type I and II receptors.

Results: We found that less P cell percentage was significantly associated with prolonged neutrophil recovery time after chemotherapy. Multivariate analysis demonstrated that P cell percentage was an independent factor of delaying recovery of neutrophils, suggesting that P cells may be affecting neutrophil recovery capability for both first and second courses (p=0.015; p=0.036, respectively).

Summary/Conclusions: Our results indicate that CD34+CD38+CD117+HLA-DR<CD13+CD33+ cells before each course of chemotherapy is associated with chemotherapeutic hematopoietic reconstitution capacity independently. These findings may help better understand endogenous hematopoietic reconstitution and modify future chemotherapy regimens based on progenitor cell percentages.

E919 MICRORNAS (miRS) IN HIGH RISK PEDIATRIC ACUTE MYELOID LEUKEMIA (AML) AS PREDICTION TOOLS FOR RELAPSE INCIDENCE

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Background: Despite recent progresses made in the treatment of acute myeloid leukemia (AML) of childhood, the cure rates of high-risk subtypes remain low. Indeed, patients harboring FLT3-ITD mutations or 11q23 translocations (MLL rearrangements) are still characterized by a poor prognosis, mainly due to leukemia’s pathogenesis. Since miRNA (miRs) are small RNA molecules controlling normal hematopoiesis whose deregulation is fundamental in leukemia’s pathogenesis, a possible role as predictors of relapse should be considered.

Aims: Our purpose is to identify, at time of diagnosis, significant miRs signatures able to predict the risk of relapse for patients with high-risk AML, such as FLT3-ITD and MLL mutated. Moreover, these signatures would help us in identifying new molecules for novel targeted therapy and to deeply characterize different deregulated pathways among FLT3-ITD and MLL rearranged patients.

Methods: A total of 20 AML bone marrow (BM) derived samples collected either at diagnosis (ND) and at relapse (RL) together with 8 healthy controls (HCs). All the presented signatures in pediatric AML carrying FLT3-ITD and 11q23 translocations were studied. Statistical analysis was performed using nSolverTM (NanoString Technologies; Seattle, WA, USA) and R-based software. All the assessed results imply a P<0.05 where not mentioned.

Results: Comparing all AML samples with HCs, we found 16 up- and 59 down-regulated miRs. Similarly, FLT3-ITD (n=11) vs HCs, showed 17 up- and 36 down-regulated miRs, while MLL-rearranged (n=9) vs HCs showed 16 and 18 down- and up-regulated miRs, respectively. A trend towards down-regulation of the whole cohort was detected and a block in miRs maturation occurring in the 2 molecular subsets was supposed. Finally, a FLT3-ITD vs MLL-rearranged analysis produced a signature in which 20 miRs were up- and 18 down- regulated, a putative signature which could characterize high-risk AML. ND vs HCs analysis identified 17 up- and 39 down-regulated miRs, confirming a tendency toward downregulation, as well as in RL vs HCs analysis, in which we found 12 and 374 up- and down-regulated miRs, respectively. RL vs ND comparison showed a total of 16 up- and 15 down-regulated miRs. In the attempt to identify a signature predictive of recurrence at time of diagnosis, we compared ND and RL samples, revealing 301 miRs that maintained their deregulation in the 2 subgroups, while 113 and 85 were uniquely found in ND vs HCs and RL vs HCs, respectively. Remarkably, miR-34a-5p (P=0.0001) was the recurrent and most statistically significant upregulated miR in both ND and RL samples. Moreover, upregulated miR-10a-5p and miR-99a-5p (P<0.0001), and downregulated miR-5p (P<0.0001) were the most statistically significant miRs in both ND and RL samples respectively, underlying putative unique elements distinguishing the two clinical subsets.

Summary/Conclusions: Our results suggest the presence of different microRNA signatures in pediatric AML carrying FLT3-ITD and 11q23 translocations ([t(9;11) and (t;10;11)]. The identifications of new targets linked to this miRs would be useful for further studies focused on finding novel target-based therapies. Interestingly, miR-34a-5p was recurrently found upregulated either in ND and RL groups, but in the comparative analysis between ND vs RL, suggesting a potential involvement in the mechanisms at the base of both onset and relapse in these subtypes of high-risk AML.
E919
MRD-DRIVEN CHOICE OF CONSOLIDATION AND MODULATION OF INDUCTION AND CONSOLIDATION INTENSITY RESULTED IN A SIGNIFICANTLY IMPROVED OUTCOME OF YOUNGER AML PATIENTS IN THE LAST THREE YEARS
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Background: In the last decades no effective new drugs have been introduced and AML induction therapy is still based on an combination of anthracyclines and cytarabine. The MRD group has, however, reported a progressive increase of cure rates in younger patients. Our group has recently showed that the outcome can be improved by a fludarabine-containing induction (FLAI5, with fludarabine administration in first course only), followed by a risk- adapted consolidation.

Aims: The aim of the present study was to evaluate if the disease free survival (DFS) and the overall survival (OS) of younger (<65 years) AML patient treated in our center had shown any modification in four consecutive periods of treatment (<2008; 2008-2010; 2011-2013; 2014-2016) and to recognize factors possibly leading to this result.

Methods: We reviewed the outcome of 145 consecutive AML patients aged 65 or less and uniformly treated according to the above mentioned strategy. Minimal residual disease (MRD) evaluation was performed by flow cytometry (MFC), assessment of WT1 expression levels and, where applicable, evaluation of recurrent abnormalities such as NPM1 mutation.

Figure 1.

Results: The cohorts of patients treated in the four periods had a comparable age and risk distribution. Notably, although the median follow up of the 4 cohorts of patients is different, patients treated in the last 3 years showed a significant improvement in DFS (Fig 1), in comparison with previously treated patients. When we reviewed our experience, we found that some changes we introduced in the therapeutic management, possibly contributed to improve outcome. Beside classical risk factors, the time from hematological recovery after the induction (induction 1) and the start of the second induction course (induction 2) proved to be significantly related to DFS and OS probability. An interval shorter than 15 days resulted in significantly higher toxicity, whereas a time longer than 26 days was associated with an increased relapse probability. Patients being treated in the last three years had a median time from recovery after induction 1 to start of induction 2 of 17 days, compared to 22 days in the other cohorts (p<0.05). Furthermore, after 2013, MRD information after induction 1 was added as a prognostic factor and ELN low and intermediate risk patient with negative MRD after induction 1 were no more scheduled for early allogeneic stem cell transplant (HSCT), but received an higher dose of Ara-C in each of the three consolidation cycles (12g/qm cumulative dose vs 8g/qm). Among 8 intermediate risk patients who were MFC MRD negative post FLAI and did not proceed to HSCT in first complete remission (CR1), only one relapsed whereas among 5 intermediate risk patients who underwent HSCT in CR1 because of MRD positivity no relapses have been observed. Starting from 2014, patient in CR1 not scheduled for HSCT who showed MRD negativity underwent salvage therapy before overt hematologic relapse, followed by HSCT consolidation. MRD-directed therapy allowed all treated patients to achieve MRD negative remission before HSCT. Finally, the improved outcome may be associated with a reduced incidence of invasive fungal infections (IFI) due to the introduction of prophylaxis with posaconazole. The lower rate of IFI contributed to the reduction in the delay between chemotherapy courses.

Summary/Conclusions: Our experience shows that, even without the contribution of new drugs, more appropriate utilization of HSCT, tailored on early MRD assessment, MRD directed salvage therapy and posaconazole prophylaxis of IFI led to a relevant improvement of outcome.

E920
EFFECTIVENESS OF TREATMENT ACUTE MYELOID LEUKEMIA IN THE ELDERLY USING CLADRIBINE WITH LOW-DOSE ARAC
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Background: Treatment of acute myeloid leukemia(AML) in the elderly, unfit patients is a challenge for clinical hematologists. Therapeutic management in this population is based on limited evidence and numerous controversies including early deaths. A standard treatment of low dose cytarabine (LD-AraC) or using hypomethylating therapy is not satisfying enough. Polish Adult Leukemia Group’s (PALG) studies showed, that addition of cladribine to daunorubicine and cytarabine increases the complete remission rate and improves overall survival in younger patients with AML. We also proved effectiveness of cladribine combined with high dose AraC and mitoxantrone in relapsed and refractory AML (1, 2). Cldaribine, enhances the concentration of Ara-CTP, an active metabolite of Ara-C in leukemic cells (3). Recent data indicate that cladribine has also hypomethylating properties.

Aims: The aim of our study was to evaluate the efficacy and toxicity of cladribine in combination with LD-AraC in older AML patients.

Methods: Patients with newly diagnosed AML (excluding APL), older than 60 years, unfit for standard induction chemotherapy, were enrolled to our study. The patients were given two cycles of cladribine 5mg/m² i.v. on days 1-5 and low-dose cytarabine 40mg/m² s.c. daily on days 1-10 every 28 days if no complications including early deaths. A standard treatment of low dose cytarabine (LD-AraC) or using hypomethylating therapy is not satisfying enough. Polish Adult Leukemia Group’s (PALG) studies showed, that addition of cladribine to daunorubicine and cytarabine increases the complete remission rate and improves overall survival in younger patients with AML. We also proved effectiveness of cladribine combined with high dose AraC and mitoxantrone in relapsed and refractory AML (1, 2). Cldaribine, enhances the concentration of Ara-CTP, an active metabolite of Ara-C in leukemic cells (3). Recent data indicate that cladribine has also hypomethylating properties.

Results: Twenty-four patients have been enrolled with median age 70 years (range 62-84). In our cohort 20 patients had newly diagnosed AML, 3 secondary and 1 therapy related AML. Cytogenetic risk: good risk 5 patients, intermediate 12, poor risk 3 patients, 4 patients were unclassified. The overall response rate (CR+PR) was 84%. 13 out of 24 (54%) patients achieved complete remission (CR) and 7 (30%) achieved partial remission (PR). Time to median number of cycles to obtain CR was 2 (range 1-3), 16% of patients did not respond to treatment. The regimen was well tolerated without 4-week and 8-week mortality. The main reason of death were: heart failure (n=2), renal failure (n=1) and progressive disease (n=4). We didn’t observe grade 3 and 4 nonhematologic adverse events. With a median time of follow-up 14 months, the median overall survival was 12 months.

Summary/Conclusions: The combination of cladribine plus low dose AraC is effective and well tolerated regimen in elderly AML patients unfit for standard chemotherapy.

E921
SMALL CUSTOMIZABLE NGS BASED TARGET CAPTURE PANELS DETECT VARIANTS IN CLINICAL SPECIMENS AT FREQUENCIES AS LOW AS 0.5%
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Background: The use of large scale hybridization panels in early stages of clinical trials for novel therapies elicits a plethora of information for targeted biomarkers. However, as therapeutic targets are further characterized large panels generate an overly broad set of data, compromising sensitivity in the selected biomarker subset. Therefore, once biomarker targets are identified, the use of smaller hybridization panels can facilitate specific variant detection by analyzing specific genomic regions of interest with greater sensitivity than larger gene panels and PCR-based assays. Modifications of laboratory methods for small scale panels allow for the maintenance of high analytic quality with finely targeted panels. Our small panels (~10kb) focus on 1-4 genes, allowing for high-multiplexing of samples on sequencers, and reduced costs/processing times without compromising accuracy.

Methods: We have developed with bioinformatics software under ISO13485 design control. The NGS target capture panels were developed with bioinformatics software under ISO13485 design control.

Background: The use of large scale hybridization panels in early stages of clinical trials for novel therapies elicits a plethora of information for targeted biomarkers. However, as therapeutic targets are further characterized large panels generate an overly broad set of data, compromising sensitivity in the selected biomarker subset. Therefore, once biomarker targets are identified, the use of smaller hybridization panels can facilitate specific variant detection by analyzing specific genomic regions of interest with greater sensitivity than larger gene panels and PCR-based assays. Modifications of laboratory methods for small scale panels allow for the maintenance of high analytic quality with finely targeted panels. Our small panels (~10kb) focus on 1-4 genes, allowing for high-multiplexing of samples on sequencers, and reduced costs/processing times without compromising accuracy.

Aims: To demonstrate the sensitivity, linearity, concordance with other assays, and clinical applications of small NGS target capture panels.

Methods: Two separate next generation sequencing-target capture assays were developed with bioinformatics software under ISO13485 design control. The panel contained 3 genes, including fms related tyrosine kinase 3 (FLT3), the second covers only CD274 (PD-L1). Libraries were made, hybridized with baits, and sequenced using the Illumina MiSeqDx. Validation was carried out by spiking in fixed amounts of mutant DNA into wild type DNA to establish the linearity and sensitivity of the assays. Sequencing libraries were generated by capturing baits from either one panel. Sequencing data was analyzed using proprietary software developed by Invivoscribe. Eight AML clinical samples were cross validated for FLT3 mutations by this small panel, amplicon based NGS assay, and capillary electrophoresis (CE) assay.

Figure 1.
Results: DNA from 24 cell lines was assessed using both panels, confirming variants previously detected using other methods. A validation was run on the 3-gen panel using a series of corroborated samples generated from cell lines containing between 0.5% and 25% variant allele frequencies for expected variants. Initial validation indicates that these small panel assays can detect mutations down to 0.5% variant allele frequencies. Assay linearity for FLT3/TKD detection from 0.25% to 12.5% or for FLT3/TKD detection from 0.5% to 25% is excellent (R²= 0.996 and 0.998, respectively). Average sequencing coverage was high, ranging from 5,265x to 7,680x. Comparison of FLT3 analysis of the small panel to amplicon based NGS assay and CE, FLT3-ITD showed complete concordance in clinical samples - and showed a strong linear relationship between detected VAFs, and detected ITD sizes. There was also complete concordance for FLT3/TKD mutations in clinical samples.

Summary/Conclusions: Small hybridization panels are cost-effective in detecting low-frequency variants from smaller subsets of genes while using far less DNA than individual PCR-based biomarker assays would require. Additionally, preliminary data shows great accuracy on clinical samples. These smaller assays focus on the most pertinent genes for a targeted therapy, and have the potential to greatly assist in understanding the molecular backgrounds of responders, super-responders, and non-responders, information which can help improve patient outcomes. Developing these assays with bioinformatics using the international ISO13485 design control standards makes them suitable for regulatory approval worldwide.

E923

MOLECULAR GENETIC TESTING PATTERNS FOR PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA (AML) ENROLLED IN THE CONNECT™ MDS/AML DISEASE REGISTRY


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Background: Recurrent mutations in AML-associated genes have prognostic value and may help guide treatment decisions. Molecular genetic testing patterns for AML in clinical practice are largely unknown. Previous results of the CONNECT MDS/AML Disease Registry (George et al. ASH 2016; abstract 3548) showed suboptimal adherence to WHO 2008 recommendations for AML diagnostics in a cohort of patients with newly diagnosed AML in clinical practice.

Aims: To report a detailed analysis of patterns of molecular genetic testing in patients with newly diagnosed AML in community and academic settings.

Methods: The CONNECT MDS/AML Disease Registry (NCT01688801) is a multi-center, observational, cohort study of patients with newly diagnosed AML (≥25 years) or myelodysplastic syndrome (MDS). All clinical decisions are made by the treating clinicians. Data are collected, using an electronic data capture system, at screening, enrollment, and approximately quarterly throughout the duration of the patient’s participation in the registry. All patients provided informed consent. Enrollment is ongoing. The current analysis evaluated the percentage of patients with AML who had undergone molecular genetic testing recommended by NCCN guidelines (NPM1, FLT3-ITD, CEBPA, IDH1, IDH2, DNMT3A, and KIT). Chi-square tests evaluated effects of several variables on likelihood of molecular genetic testing.

Results: Between 1 Dec 2012 and 8 Dec 2016 (data cutoff), 259 patients with AML were enrolled at 86 sites. Molecular genetic testing was reported in 67% (173/259) of patients. Likelihood of testing varied, respectively, for academc vs community sites (76% [70/92] vs 62% [103/167], P = .018), normal vs abnormal karyotype (77% [79/103] vs 59% [79/133], P = .006), age <65 vs ≥65 (63% [85/133] vs 55% [60/118], P = 0.003), and presence vs absence of HLA restriction (81% [83/103] vs 74% [90/122], P = .025). In patients who had undergone molecular testing (n=173), the mutations tested varied substantially. All of the NCCN-recommended molecular genetic tests were reported in 9% (15/173) of patients with 8% (6/173) being positive with normal karyotype. Of the seven NCCN-recommended tests, NPM1 (77%) and FLT3-ITD (76%) were most often reported and DNMT3A least often (16%).

Summary/Conclusions: Early data from the CONNECT MDS/AML Disease Registry reveal that despite molecular testing reported in 67% of patients with newly diagnosed AML, a majority of institutions have not developed institutional guidelines recommended testing. This prospective registry is uniquely positioned to capture changes in testing patterns as guidelines are established.

E924

PHASE 1, OPEN-LABEL, RANDOMIZED STUDY TO EVALUATE THE EFFECT OF CYTOCHROME P450 (CYP) 3A4 INHIBITION ON THE PHARMACOKINETICS (PK) AND SAFETY OF QUIZARTINIB (Q) AND ITS ACTIVE METABOLITE, AC886

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Background: Q is a potent, selective FMS-like tyrosine kinase 3 (FLT3) inhibitor currently being investigated in Phase 3 studies in AML patients (pt) with FLT3 internal tandem duplication (ITD) mutations. Early studies showed concentration (c) and area under the curve (AUC) of Q and its metabolite, AC886, were significantly reduced in patients receiving concurrent CYP3A4 inhibitors, CYP3A4. Because CYP3A4 inhibiting drugs are frequently required in the
course of AML treatment, a drug interaction study was performed to assess PK when Q is co-administered with CYP3A4 inhibitors. **Aims:** The primary aim was to determine the effect of ketoconazole (K), a strong CYP3A4 inhibitor, and fluconazole (F), a moderate CYP3A4 inhibitor, on PK of Q and AC886. The secondary aim was to assess the tolerability and safety of Q co-administered with K or F. **Methods:** This was an open-label, randomized, parallel-group study. Healthy subjects (HS) age 18-55 years (yr) who provided informed consent were randomized 1:1:1 to receive K 200mg twice daily (BID), F 200mg BID, or placebo (P) BID on Days(D) 1-28. A single 30mg dose of Q was administered to all HS on D8. Plasma Q and AC886 conc were measured D8-28, using a validated liquid chromatography-tandem mass spectrometry method. PK parameters were determined using noncompartmental analysis. Study-state (SS) drug conc, following repeated once daily dosing, were predicted using non-para-
metric superposition. An analysis of variance (ANOVA) was performed to assess the CYP3A4 inhibitory effect of K and F on the PK. **Results:** 93 HS were enrolled (31 per arm) and 88 received Q. 75% were male, median age 32 yr (18-53). Relative to Q+P, co-administration of Q+K or Q+F increased the geometric mean (Geomean) Cmax of Q by 17% and 11%, and Geomean AUC0-t by 94% and 20%, respectively (Table 1 below). The Geomean Cmax and AUC0-t of AC886 were decreased by 60% and 15%, respectively, for Q+K, and were increased by 3% and 14%, respectively, for Q+F. Apparent clearance (CL/F) of Q was 50% lower and t1/2 of Q and AC886 were 46% and 96% longer, respectively in Q+K vs Q+P. CL/F of Q was 17% lower and t1/2 of Q and AC886 were 10% and 28% longer, respectively, in Q+F vs Q+P. AC886 is a minor component in circulation relative to Q (approximately 25%). An increase of 86% in simulated SS C0-24 and 96% in SS Q AUC0-24 was predicted following repeat daily dosing of 30mg Q+K vs Q+F, while a modest decrease in AC886 exposure (<20%) was predicted. The most common (≥5%) adverse events were headache (7.5%) and diarrhea (5.4%), with the majority being Grade 1/2. There were no clinically significant hematology, clinical chemistry, QTc, or vital sign observations, and no deaths or serious adverse events. **Summary/Conclusions:** Co-administration of Q with K or F was well tolerated and safe. Overall, there was an approximate 2-fold increase in Q exposure when Q was co-administered with K, which is considered clinically significant. The increase in Q exposure when Q was co-administered with F was within 20% and is not considered clinically relevant. Given the relationship between Q conc and QTc prolongation, these results support reducing Q doses by approximately one-half when taken concomitantly with a strong CYP3A4 inhibitor. No dose reduction is needed when Q is co-administered with a moderate or weak CYP3A4 inhibitor. This approach has been implemented in two ongoing Phase 3 trials of Q in FLT3-ITD mutated AML. **E926** **CLINICAL OUTCOMES OF CHILDHOOD ACUTE MEGAKARYOBLASTIC LEUKEMIA: THE CHILDREN CANCER HOSPITAL EGYPT 57357 EXPERIENCE** N. Maarouf1,*, S. Mahmoud1,2, R. Abdelaziz1,2, L. Lehmann3, K. Shaaban4,5, S. Fahmy6,7, S. Ibrahim6,7, O. Hassanain7, N. Nader7, A. Elhaddad1,2 1Pediatric Oncology, 657357 CCHE, 2Pediatric Oncology, National Cancer Institute, Cairo, Egypt, 3Pediatric Stem Cell Transplant, Dana Farber Cancer Institute, Boston, United States, 4Department of Clinical Pathology CCHE, 57357 CCHE, 5Department of Clinical Pathology, National Cancer Institute, 6Department of Clinical Pathology, 7Department of Research CCHE, 57357 CCHE, Cairo, Egypt **Background:** Acute megakaryoblastic leukemia is a rare subtype of pediatric AML occurring in both Down and non-Down syndrome patients. Down syndrome patients with M7 subtype have an excellent prognosis while non-Down syndrome patients have poor outcomes. Heterogenous cytogenetic abnormalities have been described in M7 AML and the impact of different prognostic factors on outcomes is yet to be determined. **Aims:** To evaluate the prognostic significance of various cytogenetic abnormalities and minimal residual disease (MRD) by flow cytometry after induction I and correlate them with clinical outcomes of patients with acute megakaryoblastic leukemia. **Methods:** We retrospectively analyzed the data of 80 non-Down syndrome patients diagnosed with M7 AML treated at CCHE between January 2007 through December 2016. Three treatment protocols were used. **Results:** The median age at diagnosis was 1.7 years (range 0.2-15). The medi-
an time to diagnosis was 1 month. The overall (OS), event free survival(EFS) and cumulative incidence of relapse at 2 years were 53.4%, 42.9% and 28.4% respectively. Sixty one patients had abnormal cytogenetic abnormalities including Trisomy 19 (n=20), 13q (n=3), Trisomy 8 (n=12), Complex karyotype (n=28), t(1;22) (n=12), MLL gene rearrangement (n=9), Trisomy 21 (n=24) but none of these had an impact on outcomes. Out of the 80 patients 56 were in complete remission post induction I. Two hundred two patients had MRD<0.1% after induction I. In the univariate analysis patients with MRD <0.1% post induction I had a better OS and EFS with a lower cumulative incidence of relapse however these findings did not reach a statistical significance. **Summary/Conclusions:** Acute megakaryoblastic leukemia in non-Down syn-
drome patients have poor outcomes irrespective of any cytogenetic abnormalities. Future direction to determining tumor biology based on molecular path-
ways in this disease is being considered. **E927** **IDENTIFICATION OF RESISTANCE ASSOCIATED CPG METHYLATION CHANGES IN ACUTE MYELOID LEUKEMIA PATIENTS UNDERGOING INDUCTION CHEMOTHERAPY** C. Niederwieser1,2, C. Rohde1, H. Serve2, W. Berde3, G. Ehringer1, S. Göllner1, L. Müller1, C. Müller-Tidow1 **Background:** The identification of novel therapeutic targets in acute myeloid leukemia (AML) is limited. Genome-wide DNA methylation profiling of AML patients is an established molecular approach which can be used to identify novel therapeutic targets. **Methods:** We performed pyrosequencing of ACGT methylation in untreated AML patients undergoing induction therapy. **Results:** We identified methylation changes in 38 genes, including SMARCB1, TP53 and PTEN. **Summary/Conclusions:** These findings highlight the potential of genome-wide methylation analysis as a tool for the identification of novel therapeutic targets in AML.
Background: Acute myeloid leukemia (AML) is a heterogeneous disease associated with epigenetic alterations that can be targeted with demethylating agents to induce CR in a subgroup of patients. However, there are currently no predictive markers that reliably distinguish responder from non-responder patients. In this analysis we assessed DNA methylation changes in a group of refractory patients with AML treated either with the hypomethylating agent azacytidine followed by intensive chemotherapy or with intensive chemotherapy alone in order to identify the alterations and genes involved.

Aims: The exploration of whole genome methylation changes of azacytidine and chemotherapy treatment in refractory patients with AML guides treatment refinement.

Methods: Patients from the AML-AZA trial of the Study Alliance Leukemia were randomized to receive either azacytidine followed by chemotherapy or chemotherapy alone. Cells were harvested at baseline and 15 days after chemotherapy from 16 of the 105 patients receiving the combination and from four of the 109 patients randomized to receive chemotherapy only. Genome wide DNA methylation was analysed using a 450K Illumina array (Illumina, San Diego, USA). With a signature derived by differential blasts within diagnosis to day 15, patients with a reduction of blasts clustered together by methylation of all the selected CpG sites, as did those with an increase of blasts on both day 0 and day 15, or those paired samples of day 0 and day 15 frequently clustering together as well. This led us to refine blast independent analyses. We excluded methylation changes correlating with the percentage of blasts (p=0.14, exploratory regression among blast change and median methylation change change day 0 to day 15, each), since these are likely to reflect the increased lymphocyte counts among blast samples used for analysis. Motifs most strongly impacted by methylation changes were detected using the Homer software (Salk institute, San Diego, USA), Methylation changes were compared between the two groups to identify the changes associated with the use of azacytidine prior to chemotherapy.

Results: In the Azacytidine plus Chemotherapy treated group, a total of 389 differentially methylated regions (DMRs), most of which were single CpGs, were identified, 176 of which were hypermethylated and 213 hypomethylated. The most highly represented hypermethylated loci were KDM7A (p=1e-17, 6.25% of 176 DMRs), KLF13 (p=1e-14, 7.95%), HIC2 (p=1e-11, 5.11%), while those most commonly hypomethylated were ARF (p=1e-15, 2.82% of 213 DMR’s), MYB (p=1e-14, 3.76%) and STAT1 (p=1e-14, 1.88%). The chemotherapy alone group yielded 7181 DMRs, 5752 of which were hypermethylated and 1429 hypomethylated. The genes most commonly hypermethylated in these patients were EEF(EFS) (p=1e-226, 32.78% of 5752), CEFSB (p=1e-90, 10.34%), and Jun-AAP (p=1e-45, 6.10%), while those most commonly hypomethylated were RUNX1 (p=1e-24, 28.34% of 1429 DMRs), TCFF4 (p=1e-21, 8.40%) and SMAD3 (p=1e-17, 1.05%). Median overall survival did not differ between the two treatment groups, with 153 days for chemotherapy and 143 days for chemotherapy patients.

Summary/Conclusions: Methylation changes associated with azacytidine and chemotherapy of refractory patients were particularly found in genes previously associated with cancer and AML. DNA hypermethylation was more common after chemotherapy alone. This finding suggests that DNA hypermethylation of specific loci may be associated with therapy resistance. Hence, the methylation levels were detected from the most resistant cells. Of note, upon Azacytidine treatment more hypomethylated loci were observed. This potentially indicates DNA hypomethylation in vivo.

E928

OVER-EXPRESSION OF ZEB2-AS1 LncRNA PREDICTS POOR OUTCOMES IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) is a fatal hematopoietic malignancy with poor clinical outcomes characterized by blasts infiltrated in tissues. Aims: To determine whether the antisense IncRNA namely ZEB2-AS1 would be associated with clinical outcomes, we assessed its expression in retrospectively collected with 82 newly AML cases.

Methods: Relative quantitative real-time PCR analysis was employed for detecting levels of ZEB2-AS1. SYBR Green RT-PCR was performed, followed by obtaining relative threshold cycle normalized to reference GAPDH gene. Cell migration, invasion, proliferation and apoptosis tests were used to analyze biological phenotypes of AML cells after knocking down ZEB2-AS1 IncRNA by small interfering RNAs.

Results: Results showed that expression of ZEB2-AS1 IncRNA was prominently high and closely correlated with adverse clinical outcomes in AML patients, based on either modified MRC or ELN risk stratification system. Univariate analyses indicated that patients with higher expression of ZEB2-AS1 IncRNA had significant shorter 3-year overall survival (OS) (0% vs 68.2%, p=0.036) and disease-free survival (DFS) (25.0% vs 68.8%, p=0.039). In addition, Patients with higher expression of ZEB2-AS1 IncRNA had significant lower complete remission (CR) rate in response to induction chemotherapy (75.0% vs 27.3%, p=0.031). In patients with low levels of ZEB2-AS1 IncRNA, patients treated by allogeneic hematopoietic stem cell transplantation had significant longer OS (3-year OS, 75.8% vs 28.6%, p=0.037) and DFS (3-year DFS, 81.8% vs 26.8%, p=0.049) compared to that of chemotherapy.

Summary/Conclusions: Moreover, knockdown of ZEB2-AS1 IncRNA could effectively inhibit invasion and migration in AML cells, which was closely associated with down-regulation of ZEB2 and up-regulation of E-cadherin. Collectively, although independent prognostic value for survivals was not rigorously determined, ZEB2-AS1 IncRNA may serve as candidate to improve conventional risk stratification system and contribute to evaluating therapeutic responses. Furthermore, ZEB2-AS1 IncRNA could be a potential therapeutic target for patients with AML treated either with the hypomethylating agent azacytidine or chemotherapy alone.

E929

INTENSIFICATION OF ANTHRACYCLINE DURING INDUCTION AND CONSOLIDATION IS SAFE AND WELL TOLERATED IN OLDER PATIENTS WITH ACUTE MYELOID LEUKAEMA

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Background: AML in the elderly is more susceptible to treatment failure. Treatment related mortality in elderly patients with AML is decreasing over time, and receiving chemotherapy of adequate intensity is important in treating AML in these patients. The optimal induction and consolidation approach for patients in this age group is yet to be established, however data from the HOVON group has demonstrated the benefit of anthracycline intensification during induction in patients aged 60-65 years, while locally the Australian AML12 study demonstrated the value of anthracycline intensification during consolidation in younger adults. We have implemented a novel combination of intensified anthracycline in combination with infusional cytarabine (AraC) during induction and in combination with intermediate-dose AraC during consolidation.

Aims: To demonstrate the safety and tolerability and provide preliminary efficacy evidence for anthracycline intensification during induction and consolidation in older adults with Acute Myeloid Leukaemia.

Methods: A retrospective pilot study was done on 76 consecutive patients above the age of 55 years with newly diagnosed AML between January 2010 to June 2016 at Alfred Hospital, Melbourne, Australia. All received the 7+3 induction regime (AraC continuous infusion at dose of 100mg/m2/day on days 1 to 7, and idarubicin at a dose of 12mg/m2/day on days 1 to 3), with a planned consolidation with AraC (AraC 100mg/m2 twice daily Day 1, 3, 5, and idarubicin 12mg/m2/day Day 1-2). Outcomes were assessed according to the Cheson criteria with cytogenic risk assessed by the refined Grimwade MRC criteria.

Table 1.

Results: 76 patients, with a median age of 62 years (range 55.4-70.6 years) received the 7+3 induction with a median overall survival of 590 (range 6-1996) days and overall response rate was 52 patients (68.4%). The event-free survival median is 109 days (range 6-1988) and the relapse-free survival median is 314 days and overall response rate was 52 patients (68.4%). The event-free survival median is 109 days (range 6-1988) and the relapse-free survival median is 314 days (range 4-1947). There were 9 treatment-related deaths (11.8%) within 30 days following 7+3 induction. Of 41 patients who attained complete morphologic remission after induction, 29 patients (70.7%) received the planned IDAC+2 consolidation with 17 (41.5%) receiving two consolidation cycles. Of those not receiving IDAC+2, 10 patients (24.4%) received an alternative consolidation regimen and 2 patients (4.9%) did not receive consolidation. Of those receiving IDAC+2 25 (86.2%) were intermediate cytogenetic risk and 3 (10.7%) were favorable. No treatment-related deaths occurred following IDAC+2. 20 patients (26.3%) from the whole cohort received an allogeneic stem cell transplant (SCT), and 8 patients (27.6%) of those who received the IDAC+2 consolidation regimen proceeded to an allogeneic SCT in all IDAC+2.
consolidation cycles, the median days to neutrophil recovery was 26 days (range 18-72), platelet recovery 32 days (range 17-75), and the ICU admission rate was 12.8% (range 2-10 days). 18 patients (62.1%) receiving IDAC+2 consolidation suffered disease relapse. For patients receiving IDAC+2 consolidation the median OS was 727 days (range 113-1614 days) with an EFS of 388 days (range 109-1614 days). For patients aged 60-65 years the remission rate and survival outcomes were similar to those published by Lowenberg et al. for novel therapeutic approaches in this patient group.

**E930**

**PROGNOSTIC IMPACT OF IDH1 AND IDH2 MUTATIONS IN LOW AND INTERMEDIATE RISK AML: A MULTICENTER RETROSPECTIVE STUDY**

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**Background:** Mutations in the isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) genes are common in acute myeloid leukemia (AML) but, although investigated in several studies, their prognostic significance still remains controversial.

**Aims:** To evaluate the prevalence and prognostic impact of IDH1 and IDH2 mutations in adult AML patients with low and intermediate-1 and 2 risk (European Leukemia Net, ELN 2010).

**Methods:** We retrospectively evaluated IDH1 and IDH2 mutations in 99 low and intermediate risk patients with new diagnosed AML who underwent intensive induction chemotherapy in three Italian centers.

**Results:** Median age for all patients was 60 years. IDH mutations were detected in 31% of our patients. 7% were IDH1 R132, 16% were IDH2 R140 and 2% R172. Median WBC count was 12.6x10^9/L in IDH wild-type, and 24.7x10^9/L in IDH mutated. Absolute neutrophil count was 3.1x10^9/L in IDH wild-type and 0.9x10^9/L in IDH mutated, and the difference was statistically significant (p<0.001). Median bone marrow blasts, platelets count, and LDH did not differ significantly. Cytogenetic risk group according to ELN 2010 showed favorable risk in 31.4%, and intermediate (I and II) risk in 68.6%. In favorable risk group IDH mutated patients were 12%, and 13% in the intermediate risk group. IDH expression was significantly correlated neither with NPM1 mutation nor with FLT3 mutation. There were no significant differences between induction therapy approaches and IDH status. In the whole cohort of patients, in favor of IDH mutated patients was the 1-year overall survival (OS) rate was 43.3%, 24.2% for the refractory or relapse AML. CR was 61.0% (11/18), PR was 22.2% (4/18), and ORR was 83.3% (15/18). While for the other not getting CR after a course of induction chemotherapy, CR was 55.6% (10/18), PR was 22.2% (4/18), and ORR was 77.8% (14/18). Grade 2-4 hematologic toxicities were observed in all patients, and 72.2% cases experienced infection. And all non hematological side effects were mild and well-tolerated. With a median follow-up of 7.5 (0.5-33.3) months, the 1-year overall survival (OS) rate was 43.3%, 24.2% for the refractory or relapse AML patients, and 61.6% for those not achieving CR after a course of induction chemotherapy. The difference was significantly (P=0.01).

**Summary/Conclusions:** DAC combined with HAAG regimen is safe and effective salvage treatment for advanced stage AML patients.

**E931**

**DECITABINE COMBINED WITH HAAG REGIMEN IS AN EFFECTIVE SALVAGE TREATMENT FOR ADVANCED ACUTE MYELOID LEUKEMIA**

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**Background:** As relapsed or refractory acute myeloid leukemia (AML) are known to have poor prognosis, achieving complete remission (CR) and long-term survival become extremely challenging for these patients. Reasons for the dismal prognosis rely on more frequent resistance to conventional chemotherapy and higher treatment-related mortality. Therefore, novel therapeutical approach to treat these patients were developed.

**Aims:** To evaluate the clinical efficacy and safety of decitabine (DAC) in combination with HAAG regimen (nomaribine (KNH), cytarabine (Ara-C), doxorubicin (Acla) and recombinant human granulocyte colony stimulating factor (G-CSF)) for advanced patients with AML.

**Methods:** Thirty-six patients with advanced AML receiving DAC combined with HAAG chemotherapy in our center from December 2012 to August 2015 were enrolled in this study. Seventeen of them were refractory or relapsed AML, and another 18 patients were those who didn’t achieve CR after a course of induction chemotherapy. The therapeutic responses, side effects and long-time survival were retrospectively analyzed.

**Results:** After a course of treatment, the rate of CR and partial response (PR) was 58.3% (21/36) and 12.2% (4/36) respectively, while the overall response rate (ORR) was 80.6% (29/36) in the cohort. For the patients with refractory or relapse AML, CR was 61.0% (11/18), PR was 22.2% (4/18), and ORR was 83.3% (15/18). While for the other not getting CR after a course of induction chemotherapy, CR was 55.6% (10/18), PR was 22.2% (4/18), and ORR was 77.8% (14/18). Grade 2-4 hematologic toxicities were observed in all patients, and 72.2% cases experienced infection. And all non hematological side effects were mild and well-tolerated. With a median follow-up of 7.5 (0.5-33.3) months, the 1-year overall survival (OS) rate was 43.3%, 24.2% for the refractory or relapse AML patients, and 61.6% for those not achieving CR after a course of induction chemotherapy. The difference was significantly (P=0.01).

**Summary/Conclusions:** DAC combined with HAAG regimen is safe and effective salvage treatment for advanced stage AML patients.
Markers. Results were given overall and stratified by age (<60/≥60 years) and
sex. Kaplan Meier curves and Cox regression (Hazard ratios; HRs) was used to
compare survival by cohabitation (living with someone, living alone) and
marital status (married, divorced, widowed, unmarried).

Results: The study included 3243 AML patients. Patients living with someone
(n=2056) were younger, more likely to be married, male, to be working, and
have a higher education than patients living alone. Comorbidity, white blood
cell count, lactate dehydrogenase, and blast counts did not differ between
groups, however patients living with someone tended to have better perform-
ance status at time of diagnosis. Patients living with someone were more likely
to receive intensive chemotherapy than patients living alone when aged 60
years or older (41.2% versus 22.8%, adjusted HR 0.81 (CI=0.46-0.81)). In patients
<60 years, never-married patients were less likely to receive intensive therapy
(adjusted OR 0.43 (CI=0.19-0.99)) than married patients. In patients
<70 years achieving CR, the chance of alloHCT was reduced when living
alone (11.8%, adjusted OR 0.47 (CI=0.28-0.78), versus 19.0% in patients living
with someone). In diverse cohabitation, the chance was also reduced (7.6%
adjusted OR 0.38 (CI=0.20-0.74)) compared to married patients (19.3%). Crude
survival by cohabitation is shown in Figure 1. Overall survival was inferior in
patients ≥60 years living alone (adjusted HR 1.21 (CI=1.09-1.33)) and unmar-
ned patients (never-married: adjusted HR 1.29 (CI=1.08-1.57), divorced/wid-
owed: adjusted HR 1.11 (CI=1.00-1.23)) compared to married patients. In con-
trast, cohabitation and marital status did not affect treatment response (living
with someone: CR 70.6%, living alone: CR 72.8%) or overall survival (adjusted
HR 1.08 (CI=0.81-1.23)) in intensive therapy patients only.

Summary/Conclusions: Our study results indicate, that the effect of cohabi-
tation and marital status on AML outcome, especially in patients ≥60 years, is
explained by social support rather than by differences in income and occupation.
Patients living alone do not present with more advanced disease or higher
comorbidity burden than patients living with someone. Still, patients living alone
and never-married patients are less likely to receive intensive chemotherapy
affecting overall survival. Increased focus on what drives treatment decisions
in patients lacking social support is important to improve survival in these patients.

E933
TREATMENT OF MOLECULAR RELAPSE IN ACUTE MYELOID LEUKEMIA WITH MUTATED NPM1 REDUCES TOXICITY OF SALVAGE TREATMENT AND IMPROVES DISEASE CLEARANCE
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Background: Acute Myeloid Leukemia with mutated NPM1 (NPM-AML) is char-
acterized by a favorable prognosis. Most patients achieve complete remission
(rem) and may proceed to allogeneic hematopoietic stem cell transplantation
(SCT) or to a 2nd induction (ind) chemotherapy course aiming at achieving remis-
sion (CMR). Our aim was to further evaluate the role of NPM1 mutated leukemia
(NPM-MLD) in the context of hematological relapse (HR) and treatment of drug
resistant leukemia.

Methods: The study was a single center, prospective, open-label, single
arm, non-randomized study, comparing NPM-MLD in hematological relapse
(HR) and treatment of drug resistant leukemia. The study included all con-
msecutive patients with NPM-MLD (n=2056) who were not eligible for further
allogeneic SCT or a 2nd induction chemotherapy course. The primary end point
was achievement of remission (CR) and the secondary endpoints were the
percentage of patients with CR after the 2nd consolidation chemotherapy
course and survival at 2 years.

Results: Among 36 patients, 13 showed CR, achieved in 4/13 patients (31%). Starting from January 2015, 4 patients who
met the MRD relapse criteria received preemptive therapy, consisting of a
single course of MEC. Four consecutive patients have been treated so far. Pre-
chemotherapy and post-therapy disease burden, assessed by NPM levels, was
significantly lower than in patients treated in HR (p<0.001, Figure 1). Both
hematological and non-hematological toxicity was significantly lower than in
patients treated in HR. Notably, all patients were able to achieve complete MRD
clearance before HSCT and are alive and well at the time of the analysis.

Figure 1.

Summary/Conclusions: Despite the good overall prognosis, a significant pro-
portion of NPM-AML patients will relapse. Our preliminary data strongly support
the feasibility and efficacy of MRD-directed therapy in NPM-AML. This strategy
reduces the toxicity related to re-induction and increases the proportion of
patients achieving a MRD negative CR.

E934
MINIMAL RESIDUAL DISEASE AND LAIP CHANGES BY FLOW CYTOMETRY IN DE NOVO ACUTE MYELOID LEUKEMIA DURING CHEMOTHERAPY AND CLINICAL OUTCOMES
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Background: Minimal residual disease (MRD) detection by multicolor flow
cytometry (MFC) in acute myeloid leukemia (AML) is widely explored by differ-
ent researchers and it is an additional independent factor in clinical outcomes.
The prognostic value of leukemic associated immunophenotype (LAIP) changes
in patients with AML is not yet explored.

Methods: In a clinical prospective study since March 2016 till February 2017 50
patients (pts) de novo AML (f/m 32/18 m. age 44 (17-85)) were included. 14 pts
by this moment completed basic chemotherapy (ChT) courses: 7±3 other in-
duction and 2 consolidation. Among them favorable cytogenetics was in 4pts
(t(16;21)-1, 16q22-1, t(8;21)-2pts), intermediate-7 (6-w with normal cytotype, t-
(17;22)), poor-3 (complex karyotype-2, 11q23-1pt). Bone marrow samples were
studied in standardized panel with most common antibodies by 6-color
MFC (BD FACSCanto II, USA) before the treatment, after 1st and 2nd
courses of induction and after 2nd consolidation. Any amount of MRD >0 was
assumed as MRD positivity. Besides MRD status we also explored LAIP changes
in patients with MRD >0 before and after 1st ChT and after 2nd ChT.

Results: Leukemia associated immunophenotype (LAIP) was detected in all
monitored patients at the diagnosis. Molecular markers were detected in 28.5%
(2pts-with NPM1+FLT3+CEBPA+, 1-w ith FLT3+, 1-NPM1+), 2 pts had resistant
AML after 2 courses (CR). 3 pts out of 7 with complete morphological remission
(CMR) after 1st course had MRD positivity (0.03%, 1.61%, 8.3%), and these pts
were CRD-negative after 2nd course. CMR was achieved after 2nd course in
5 more pts and MRD positivity was detected in 3 pts (0.033%, 0.523 and 3.9%)
with intermediate cytogenetic risk. By the end of 4th course 11 pts stayed in
CRM and we diagnosed 1 morphological relapse (patient with MRD-negativity
and CMR after 2nd ChT). Two early relapses were also noticed: both with persist-
ent MRD during all period of ChT and CMR after the second ChT. All pts with
MRD-negative status after first course are alive and in CRM (8 months from
diagnosis). While monitoring, LAIP changes were distinguished in 7 pts. One
from two with resistant AML lost CD65, another one acquired CD11b. 5 pts were
in CMR after the second course and during ChT one of them gained CD56 and
CD13, 2nd, lost CD65 and CD11b, 3rd – gained CD65, 4th gained CD11b after
2nd ChT, the last one didn't change LAIP. We detected relapse in 3 pts from this
group and one – with increasing MRD after 4th course and cytopenic syndrome.
We may suggest that LAIP changes during ChT reflect selection of more
hemoresistant leukemia clone, followed by subsequent relapse.

Summary/Conclusions: 1. The most favorable group consisted of MRD neg-
ative pts after 1st course. LAIP changes are common in pts with less favorable
prognosis.
Background: New drug combinations and higher intensity therapy have led to significant improvements in complete remission (CR) rates for patients with acute myeloid leukemia (AML). However, relapsed disease remains a major source of failure. With the exception of allogeneic stem cell transplant (SCT), there are few options for post-consolidation maintenance of remission in high-risk patients. NK cells as part of the immune microenvironment are important mediators of immune surveillance in AML. Lenalidomide has demonstrated single-agent activity in AML and enhances NK cell activity and immune synapse formation in leukemia.

Aims: We designed a phase II clinical trial studying the efficacy of lenalidomide as maintenance therapy in AML patients with high-risk disease in remission, who were not being considered for SCT.

Methods: AML patients ≥18 years with a high-risk feature in 1st CR (CR1) or any patient in 2nd CR (CR2) who had received induction and at least 1 consolidation cycle were eligible for enrollment. Patients should be within 12 months of achieving CR, have PS ≤3, adequate kidney/liver function, ANC >0.5 and platelets ≥30. Patients were treated continuously with lenalidomide 10mg PO daily on D1-28 of a 28 day cycle for up to 24 cycles. All pts had baseline cytogenetic and molecular testing, and minimal residual disease (MRD) assessment by flow cytometry. After cycle 1, stepwise dose escalations were allowed to 20mg daily in pts who were tolerating their dose and have presence of minimal residual or morphologically detectable disease.

Results: A total of 14 patients have been enrolled with a median age of 57.5 years (range, 23-67). All pts were in CR at the time of enrollment, with 12 pts (86%) in CR1 and 2 (14%) in CR2. Baseline pt characteristics are outlined in Table 1. AML-related mutations detected at start of therapy include: CEBPA (n=5), NPM1 (3), FLT3 (3), IDH2 (2), NRAS (2), DNMT3a (2), and 1 each of JAK2, TET2, and EZH2. High risk features at the time of enrollment were as follows (some are overlapping): S (36%) with history of prior myeloid neoplasm or therapy related AML, 4 (29%) persistent MRD, 4 (29%) adverse mutational profile, 2 (14%) adverse karyotype, 1 (7%) primary refractory disease, and 2 (14%) CR2 status. Patients have received a median of 9 cycles (1-24) cycles of therapy. With a median followup of 19+ months (8.5-39), the 6- and 12-month estimated RFS were 100% and 69%, respectively. The 6- and 12-month estimated OS were 100% and 90%, respectively (Figure 1). The regimen was well tolerated. Cytopenias were mild and managed with dose adjustments. The most common grade 3 (no grade 4 toxicity) non-hematotoxicities were 1 each of rash, fatigue, cough, and nausea, vomiting, and stroke.

Table 1.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median (range)</th>
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</thead>
<tbody>
<tr>
<td>Age</td>
<td>58 (18-74)</td>
</tr>
<tr>
<td>WBC (10^9/L)</td>
<td>4.5 (2.3-9)</td>
</tr>
<tr>
<td>Platelets (10^12/L)</td>
<td>119 (71-215)</td>
</tr>
<tr>
<td>LDH</td>
<td>159 (20-1298)</td>
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<tr>
<td>Albinera</td>
<td>42.1 (33-47)</td>
</tr>
<tr>
<td>Creatinin</td>
<td>0.6 (0.2-1.1)</td>
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<td>Creatinina</td>
<td>0.6 (0.4-1.2)</td>
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Summary/Conclusions: Lenalidomide is a safe and feasible maintenance strategy in high-risk AML patients who are not candidates for SCT. The study continues to surpas the pre-specified expected rate of relapse-free survival of high-risk patients based on a historical cohort. Studies evaluating dynamics of MRD on study are ongoing.

E936 POSTREMISSION THERAPY FOR AML WITH INTERMEDIATE RISK CYTOGENETICS IN FIRST COMPLETE REMISSION

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Background: Postremission therapy of AML with intermediate risk cytogenetics in first CR is based on chemotherapy with high dose cytarabine (HIDAC) or hematopoietic cell transplantation (HCT). Evidence from single trials with regards to optimal postremission therapy has been inconclusive, metaanalyses suggest a survival benefit of allogeneic HCT in first CR, except for patients with mutation of NPM1 without concomitant FLT3/ITD.

Aims: We analyzed retrospectively data from patients with AML with intermediate risk cytogenetics in CR1 with the aim to determine rates of completion of postremission therapy, rates and risk factors for early relapse and non relapse mortality (NRM), overall survival (OS) and relapse free survival (RFS) according to postremission treatment and describe causes of and risk factors for treatment failure.

Methods: Data on 304 patients in CR1 treated with curative intent between 2007 and 2016 in four centers participating in Czech Leukemia Study Group for Life were analyzed. All patients signed informed consent with data collection, analysis and publication. Cox regression was used to determine risk factors for OS and RFS, using time dependent covariates for postremission therapy. Age, WBC count, number of induction cycles, NPM1 mutation, FLT3/ITD, performance status, BMI, previous malignancy and extramedullary disease were included in models. Postremission therapy was completed after HCT or after three cycles of HIDAC or HCT in patients <= 60 years and two cycles of intermediate dose cytarabine (IDAC) in patients >60 years. Competing risk cumulative incidence estimates were calculated for NRM and relapse. Early relapse and NRM were defined as relapse/NRM before completion of postremission therapy.

Results: Median age was 52 (18-74) years. Median follow up time was 481 (31-3364) days. Early relapse rate (RR) and NRM were 11.01% and 5.29%, respectively. Median OS after early relapse was only 128 days. Presence of FLT3/ITD mutation and high body mass index were associated with increased risk of early relapse on multivariate analysis (HR 14.88, 95%CI 3.24-68.43 and 2.34, 95%CI 1.3-4.2, respectively). Age increased risk of early NRM (HR 5.13, 95%CI 1.5-17.58 for age 55-35 years). 76% of patients completed therapy: 42% received allogeneic HCT in CR1, 21% completed three cycles of HIDAC and 13% completed two cycles of IDAC. 3-year OS and RFS of the whole cohort were 53.68% and 40.26%, respectively. OS was 67% in a group of patients who completed HIDAC, 34% in IDAC group and 64% in HCT group (p=0.28469). Cumulative incidence of NRM and RR 3 years after completion of therapy were 23% and 20% after HCT, 7.13% and 51% after HIDAC and 16.8% and 66.4% after HCT, respectively, differences among groups were significant (p=0.00947 and p<0.00001). HCT reduced the risk of relapse in comparison to chemotherapy (HR 0.51, 95%CI 0.3-0.85). RFS was adversely influenced by concomitant FLT3/ITD/NPM1 mutation (HR 2.17, 95%CI 1.06-4.45). Increasing age had negative effect on OS (HR 1.65, 95%CI 1.13-2.42 for age 55-35 years). After HCT, HLA mismatch and TBI based myeloablative conditioning were associated with increased NRM (HR 6.32 (95%CI 1.89-21.14) and 6 (95%CI 1.86-19.2), respectively) in comparison to transplantation from HLA matched donors and busulfan based myeloablative conditioning.

Summary/Conclusions: The majority of patients within intermediate cytogenetic group in our analysis received allogeneic HCT. Patients who relapsed before completion of treatment had dismal outcome with very short OS. Allogeneic HCT decreased risk of relapse but led to increased NRM, reducing positive effect of HCT on OS. Risk of NRM was increased after TBI based myeloablative conditioning and after HCT in mismatched unrelated donors.

Supported by Ministry of Health of the Czech Republic, grant nr. 15-25800A. All rights reserved.
LONG TERM FOLLOW UP OF PATIENTS OVER 60 YEARS TREATED WITH INTENSIVE CHEMOTHERAPY FOR ACUTE MYELOID LEUKEMIA AND MYELODYSPLASTIC SYNDROMES...

Background: More and more data on patients over the age of 60 years treated with intensive chemotherapy are emerging, however, long term data with patient outcomes after the initial 2-5 years are lacking. In 2007, we published a single center study on patients over the age of 60 years, suffering from acute myeloid leukemia (AML) or high risk myelodysplastic syndrome (MDS), treated with intensive chemotherapy (Knip et al. Cancer 2007, 110:345-52). We now present long term follow up data of these patients, the first patient being treated in 1991, meaning 26 years ago.

Aims: To characterize the longterm outcome of elderly AML and high risk MDS patients treated with intensive chemotherapy after the usual 2-5 year follow up period.

Methods: We treated 160 patients aged 60 years or more suffering from high risk MDS and AML with intensive chemotherapy regimen between 1991 and 2004. None of the patients underwent allogeneic stem cell transplantation afterwards. We now performed a follow up of the surviving patients 10 years after publication of the initial study.

Results: In the initial study median survival from the start of induction therapy was 9.5 months (10 days to 157 months), with the median survival from diagnosis of 14 months (1 day to 157 months). At publication of the study in the year 2007, 20 patients were still alive, 18 of them presented with a low risk karyotype. 13 of these patients were in complete remission and 7 patients had relapsed. Since then 11 of the 13 patients who were in CR relapsed and died of their leukemia. One patient died of other causes and only one patient is still alive and well, currently at the age of 84. This patient initially presented with a normal karyotype, too. As a result the rate of long term survivors 5 years after treatment is 5.6% only.

Summary/Conclusions: Long term follow up data of elderly patients treated for AML and MDS with intensive chemotherapy is scarce. Our data show, that induction chemotherapy not followed by allogeneic stem cell transplantation does not result in a meaningful improvement of outcome. In addition, morbidity and lack of quality of life has to be taken into account. More data and studies on this subject are urgently needed in an aging population. In our population of 160 treated patients, 158 died of their leukaemia, only one patient died of another cause and only one single patient is still alive and well over a decade later.

FLAG-IDA FOR RELAPSED/REFRACTORY ACUTE MYELOID LEUKAEMIA: A SINGLE CENTRE 5-YEAR STUDY

Background: The treatment of relapsed/refractory Acute Myeloid Leukaemia (AML) remains a formidable challenge as the therapeutic options are limited. The regimen most commonly used in this setting, FLAG-ida (Fludarabine, cytarabine, G-CSF and idaurubicin) is considered more toxic than standard Daunorubicin plus Cytarabine (DA) regimen, often associated with prolonged periods of bone marrow suppression and predisposition to severe infections.

Aims: In this study, we present a single tertiary centre experience in the use of this regimen with a view to identifying predictive factors for survival following FLAG-ida chemotherapy. The secondary aim of this project was to assess its efficacy and safety profile in the routine clinical setting.

Methods: We conducted a retrospective chart review of patients treated with FLAG-ida chemotherapy regimen for relapsed or refractory acute myeloid leukaemia (including secondary AML) between 2011 and 2016 in a large tertiary hospital. Patients treated with FLAG-ida as first line therapy were excluded.

Results: Fifty-four patients met the criteria for inclusion in this study. The median age of the patients was 53 (10-69) years. Eighteen percent (18%) received FLAG-ida for primary refractory AML while the remainder were treated having relapsed after at least 1 previous regimen. The median time to relapse was 15 months. Complete remission was achieved in 70% of patients and 81% of these patients proceeded to have an allogeneic stem cell transplant. The median overall survival following FLAG-ida chemotherapy was 16 months with 1-year and 2-year survival rates of 59% and 46% respectively. Approximately 6% therapy-related mortality was observed. The median overall survival in patients with early relapse (<12 months) was significantly shorter than those with late relapse (>12 months): 6 months and 20 months respectively (log-rank test p value: 0.04) (Figure 1). Complete remission rates were similar between relapsed and primary refractory AML patients.

Summary/Conclusions: FLAG-ida is an effective salvage regimen in patients with refractory or relapsed AML allowing the achievement of complete remission in the majority of cases. In this single-centre cohort, early relapse, within 12 months, from first line therapy was associated with an inferior survival following salvage therapy with FLAG-ida.
DRUG-DRUG INTERACTION POTENTIAL OF GILTERITINIB IN HEALTHY SUBJECTS AND PATIENTS WITH RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA


Summary/Conclusions: While the treatment options for elder AML patients have been limited, our real world data suggest that decitabine could be an effective treatment of choice also in Asia

Results: In healthy subjects, gilteritinib exposure (expressed as Cmax and AUC24) was higher (2.2-fold increase) in subjects who were coadministered gilteritinib with a strong CYP3A4 inhibitor (ITZ) than in subjects who were administered gilteritinib alone. Coadministration of gilteritinib with RIF, a strong CYP3A4 inducer, resulted in an approximate 70% decrease in gilteritinib exposure in healthy adult subjects (Figure 1). In patients with R/R AML, midazolam exposure was approximately 10% higher when administered with gilteritinib compared to midazolam alone as reflected by the geometric mean ratio and 90% confidence intervals of midazolam Cmax (111.64%; 69.54%–179.25%) and AUC24 (109.46%; 49.82%–240.48%). Additionally, a 2-fold increase in gilteritinib exposure was observed in patients who were taking concomitant medications that were moderate or strong CYP3A4 inhibitors relative to patients who did not use a CYP3A4 inhibitor. The increased exposure in these patients, however, did not translate to differences in the incidence of drug-related safety events when compared across groups.

Summary/Conclusions: These data suggest limiting concomitant use of strong CYP3A4 inducers such as rifampin with gilteritinib, Furthermore, these data suggest coadministration of CYP3A substrates with gilteritinib is unrestricted. A comprehensive review of safety data in patients with R/R AML did not suggest that dose adjustment is warranted when gilteritinib is coadministered with strong CYP3A4 inhibitors. Although concomitant use of gilteritinib with strong CYP3A4 inhibitors (eg, ITZ or FLZ) may be permissible, precaution is warranted.

E941
A FLUDARABINE-BASED ACUTE MYELOID LEUKEMIA INDUCTION IS WELL TOLERATED UP TO 75YS OF AGE ALLOWS EARLY CONSOLIDATION AND LONG TERM SURVIVAL. A SINGLE CENTRE EXPERIENCE OF 136 CONSECUTIVE PATIENTS

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Background: For decades no effective new drugs or better anthracyclin cytara- bin combinations other than the standard 3 + 7 regimen have been available for AML induction treatment. Fludarabine-based regimens have shown good efficacy in relapsed patients but raised concern about toxicity in the induction setting (Burnett JCO 2013, PMID 23940227) a modified regimen has shown better tolerance and good results in patients younger than 60 years (ys) (Guolo AJH 2016, PMID 27084986)

Aims: We report a single center, real life experience of unselected 136 consecutive AML patients treated since 2002 in our center with Fludarabine, Aracyn, Idarubicin with or without Etoposide: FLAIE up to 65ys or FLAI up to 75ys.

Methods: Patients were treated with the FLAIE or FLAI regimen followed by Idarubicin plus Aracyn as 2 step induction. Exclusion criteria for treatment were: acute promyelocytic leukemia, poor performance status and severe comorbidity. Post remission treatment included up to three cycles of high dose Aracyn, autologous (Auto) or allogeneic (Allo) stem cell transplantation according to cytogentic and molecular risk stratification (CMR, Döhner Blood 2010 PMID 19880497) aiming for a curative strategy for all our AML patients.

Results: Median age at diagnosis was 55ys (18-75ys), median follow up was 18 months (range 3-172 months), 75% of patients (102/136) had de novo AML with strong CYP3A4 inducers such as rifampin, mostly from myelodisplastic syndrome. 19% of patients (26/136) had good CMR risk disease, 45% of patients (61/136) had intermediate risk and 36% of patients (50/136) had high risk disease. Complete remission (CR) rate was 68% and was comparable to the majority of pub-
lished trial data, considering the proportion of high CMR risk (36%) and leukemia of secondary origin (25%) and the relatively high median age: 36% of patients (49/136) were above the 60s old age limit of most AML protocols. In multivariate analysis CR rate was significantly affected by age below 50ys: p=0.011; good/intermediate CMR risk: p=0.011 and de novo AML: p=0.008. The induction death rate was 4% in line or slightly lower than published results, showing that this treatment was as well safe in low CMR group. An overall mortality was 9.6% allowing to proceed to consolidation in more than 70% of CR patients. Overall 80/136 patients (59%) were beyond 50ys, intensive consolidation with Allo or Auto was done in 34/80 patients (43%) confirming the feasibility of this therapeutic strategy. The Kaplan-Meier median probability of overall survival (OS) of the whole cohort was 28 months and factors significantly affecting were OS were age below 50ys p<0.0001; de novo AML p=0.003; good/intermediate CMR risk p<0.0002; intensive consolidation with Allo or Auto transplant p=0.0001 compared to chemotherapy alone. The mean probability of Leukemia free survival (LFS) was 88 months (median not reached). Patients above 50ys had worse OS compared to patients with low CMR (P<0.05), but no difference was found in LFS (P=0.11). The median probability of OS and LFS were 16.4 and 23.4 months respectively, this compares favorably with many published results. Chen Medicine 2016 PMID: 27472687 reported a median OS of 10.3 months in a large cohort of patients of similar age treated with intensive induction. Moreover we did not found a significant difference between the 50-59ys and 60-75ys age groups: median OS was 20.8 and 14 months (p=0.02) and median LFS was 15.9 and 23.6 months (p=0.71) respectively.

Summary/Conclusions: In our real life experience the FLAIRE/FLAI regimen combined with intensive consolidation demonstrated good long term results both in terms of OS and LFS in patients younger than 50ys, this regimen was also approved in patients beyond 60ys (P<0.05). Clinical characteristics of 60ys a difficult population to treat with a curative intention mainly because of concern of high toxicity of intensive induction regimens and higher incidence of poor risk prognostic factors.

E942
OVEREXPRESSSION OF SOX4 CORRELATES WITH POOR PROGNOSIS OF ACUTE MYELOID LEUKEMIA
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Background: The SOX4 belongs to the SOX (Sry-related high-mobility group box) family and has been characterized as a transcription factor. Over the past decade, multiple functions of SOX4 have been unveiled, and the protein is now known to play important roles in embryonic development, cell fate decision, and cellular differentiation. Overexpression and amplification of SOX4 have been implicated in various cancers and are correlated with poor prognosis. In mouse models, previous studies demonstrated that the upregulation of Sox4 can be induced by and then cooperate with the aberrant expression of AML-1-ETO, NUP98-DDX10, and PML-RARα; the overexpression of HOXA9, CREB, and Evl and the haplosufficiency of PU.1 to trigger leukemogenesis. Furthermore, a previous study that employed retroviral transduction of Sox4 and bone marrow transplantation techniques revealed that increased Sox4 expression may cooperate with the derepression of Mef2c expression to induce myeloid leukemia in recipient mice. Sox4 gene was also reported to be as a direct target of C/EBPα. C/EBPα is known to inhibit the self-renewal of leukemic cells and to restore cellular differentiation. The overexpression of Sox4 that results from C/EBPα inactivation contributes to the development of a type of leukemia that is characterized by a distinct leukemia-initiating cell (LIC) phenotype. This further indicated that Sox4 is a key oncogenic target and critical mediator of C/EBPα mutants in acute myeloid leukemia (AML), which suggests a potential novel therapeutic approach to the treatment of this disease. However, the clinical implications of Sox4 expression and its role of AML leukemogenesis are not well understood.

Aims: To further investigate the relationship between bone marrow (BM) SOX4 expression and clinicopathological parameters of de novo AML and to evaluate the prognostic value of SOX4 expression for AML patients.

Methods: From Mar 2009 to Dec 2011, a total number of 112 adult AML patients were enrolled in this study. This study was approved by the Institutional Review Board (IRB) of the National Taiwan University Hospital (NTUH) and written informed consent was obtained from all participants in accordance with the Declaration of Helsinki. Immunocytochemical staining was used to assess SOX4 expression in bone marrow leukemic cells. All statistical analyses performed for this study involved two-tailed Student’s t-tests, Mann-Whitney U tests, and Chi-square tests using SPSS 34.0 software (baseline characteristics and the correlation analysis). The correlation analysis with Cox proportional hazards regression models. Kaplan-Meier estimation techniques were used to plot survival curves and log-rank tests.

Results: We divided AML patients into two groups according to the intensity and extent of SOX4 expression as follows: low expression group (score 0-2, n=86); high expression group (score 3, 4, 5, 60), respectively. The various characteristics of patients with low SOX4 expression were presented in Table 1. In clinical manifestations of AML did not show significant differences in terms of SOX4 expression. However, AML patients with low SOX4 expression tended to have favorable-risk cytogenetic (P=0.0866). We did not observe significant differences between the high and low expression groups in terms of age, gender, hemograms, NPM1 mutation and FLT3/ITD. Additionally, of the 112 AML patients that underwent conventional intensive induction chemotherapy, 85 (75.9%) achieved complete remission (CR), and the high and low expression groups showed similar probabilities of achieving first CR (36/50, 72% vs 49/62, 79%, P=0.3219). However, high SOX4 expression were associated with increased death risk when compared to low SOX4 expression (19/38, 50% vs 30/74, 40%, P=0.2202). Furthermore, with a median follow-up period of 46.7 months (range: 0.3 to 70.9 months), SOX4 expression was associated with overall survival (OS) and disease-free survival (DFS) in all patients with de novo AML (P=0.006 and P=0.013, respectively), patients with non-M3 subtypes (P=0.001 and P=0.011, respectively), patients with intermediate-risk cytogenetics, (P=0.001 and P=0.005 respectively), or even in those with normal karyotype profile (P=0.022 and P=0.111, respectively). In multivariate analysis, high SOX4 expression was found to be an independent poor prognostic factor of OS (RR 1.924, 95% CI 1.020-3.628, P=0.043) irrespective of age, WBC count and karyotype profile and NPM1/FLT3-ITD status. A meta-analysis that we conducted using an on-line data cohort retrieved from PrognoScan (a database for meta-analysis of the prognostic value of genes; http://www.abren.net/ PrognoScan/) revealed similar findings.

Summary/Conclusions: In the current study, we found that AML patients with low SOX4 expression had higher remission rates and longer overall survival than those with high SOX4 expression, regardless of age, WBC count at diagnosis, karyotype profile and NPM1/FLT3-ITD status. Our results also reveal that SOX4 is an independent prognostic factor of AML. In conclusion, we reveal that BM SOX4 expression could serve as an informative new biomarker for the clinical diagnosis of AML patients.
mal and minimal platelet count after chemotherapy. 3. rhTPO might shorten the days of platelet count recover to at least 20×10^9/L from its nadir. The incidence of side effects were similar in both groups of the study.

Table 1.

<table>
<thead>
<tr>
<th>Control group</th>
<th>Study group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean number of platelet count</td>
<td>20.8</td>
<td>22.1</td>
</tr>
<tr>
<td>Mean days of platelet count recovery</td>
<td>5.8</td>
<td>6.2</td>
</tr>
<tr>
<td>Mean number of platelet count at 10 days</td>
<td>7.8</td>
<td>8.2</td>
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<tr>
<td>Mean number of platelet count at 15 days</td>
<td>12.2</td>
<td>12.6</td>
</tr>
<tr>
<td>Mean number of platelet count at 20 days</td>
<td>17.8</td>
<td>18.2</td>
</tr>
</tbody>
</table>

Summary/Conclusions: rhTPO, administered as dose of 15000u/day when platelet count less than or equal to 50×10^9/L, might improve the recovery of thrombocytopenia of patients with acute myeloid leukemia in CR after consolidation chemotherapy. While there was no significant difference between study group and control group, there was a decreasing trend of platelet trans fusion number and shorter time required for platelet transfusion for patients in study group.

E944

TREATMENT-ASSOCIATED SURVIVAL RATES IN OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML): A SYSTEMATIC LITERATURE REVIEW

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Background: AML patients ≥60 years old are more likely to experience complications following intensive induction chemotherapy and are at higher risk of unfavorable outcomes compared with younger patients. Information regarding optimal treatment approaches for older AML patients is limited.

Aims: Summarize outcomes associated with therapies among older AML patients, with a focus on treatment patterns and overall survival (OS) as reported in the literature.

Methods: Searches were conducted in Medline and Embase (Jan 2014–May 2016) and supplemented by conference abstracts (2015–2016). Eligibility included studies in English reporting on treatment regimens and outcomes associated with older AML patients or subgroups thereof, and conducted in the US, EU 5 (United Kingdom, Germany, France, Spain, Italy), or Japan. Only studies enrolling ≥50 patients were included.

Results: Twelve studies (19 publications) reporting on OS among older AML patients were included. Participants in most studies were newly diagnosed with AML; ages ranged from 60 to 93 years. Five non-comparative studies examining the effects of various treatment modalities were identified. Median OS in studies examining azacitidine (AZA) ranged from 10 to 12 months, whereas in studies examining induction chemotherapy or reduced intensity conditioning hematopoietic stem cell transplantation, the median OS ranged from 6.5 months (95% CI: 3.7–13.5) to 16.4 months (95% CI: 12.6–24.6), respectively. Six comparative observational studies assessed the efficacy of different treatment regimens. Intensive chemotherapy (IC) was generally associated with longer median OS compared to other regimens. In one study, median OS for patients receiving IC, lower-intensity therapy (low dose cytarabine [LD-AraC])- (AZA, decitabine), or best supportive care (BSC) was 12.4 months (95% CI: 8.5–17.4), 11.5 months (95% CI: 9.2–13.9), and 2.6 months (95% CI: 1.9–3.1), with 3-year OS rates at 27%, 17% and 6% (p<0.0001), respectively. Another study assessed the efficacy of LD-AraC relative to IC, hypomethylating agents (HMA), and BSC. Patients appeared to have longer OS when receiving IC compared to LD-AraC (median OS: 12.4 vs 9.6 months; 3-year OS: 27% vs 12%; p=0.07), and those receiving LD-AraC compared to BSC had significantly improved OS (median: 9.6 vs 3.4 months; p=0.001). In this same study, while OS was longer with HMA than LD-AraC, this difference was not significant (median OS 16.1 vs 9.6 months; 3-year OS 22% vs 12%; respectively; p=0.1). Two studies assessed the efficacy of AZA vs moderate-IC, LD-AraC, or palliative therapy, alone or in combination. AZA had a significantly better survival rate vs LD-AraC in poor prognosis patients (p=0.015). Furthermore, 1-year survival was higher for AZA-treated patients (67.8%) compared to those not treated with AZA (36.9%) (p=0.004). The efficacy of AZA relative to other conventional care regimens (CCRs) including BSC, LD-AraC, or standard IC was also examined in a randomized clinical trial (n=488). Median OS at 1-year was significantly higher for AZA relative to CCR (10.4 vs 6.5 months). Results also showed that 1-year median OS was higher with AZA than CCR in all cytogenetic risk groups, normal risk (14.1 vs 10.0), intermediate risk (13.0 vs 10.1), and high risk (6.4 vs 3.2), respectively.

Summary/Conclusions: Among older AML patients, IC tended to be associated with improved OS compared with other CCRs. However, evidence from this review indicates that AZA could be an alternative treatment option for older AML patients, whether fit or unfit for IC.

E945

SYSTEMATIC REVIEW OF HEALTH STATE UTILITY VALUES FOR ECONOMIC EVALUATION OF ACUTE MYELOID LEUKEMIA

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Background: Cost-utility analyses undertaken to inform decision making regarding acute myeloid leukemia (AML) require a set of health state utility values (HSUVs) so that the time AML patients spend in different health states can be aggregated into quality-adjusted life-years (QALY).

Aims: This study reviews AML-related HSUVs that could be used in economic evaluation and assesses their advantages and disadvantages with respect to valuation methods used and AML clinical pathways.

Methods: Embase, MEDLINE, Cochrane database, and conference abstracts (ASCO, ESMO and ASH) were systematically searched from Jan 2000 through Nov 2016 for relevant studies that reported quality of life (QOL) and HSUV in AML. Identified relevant EORTC Quality of Life Core Questionnaire QLQ-C30 values were mapped to HDUV using previously published algorithm by Crott, et al. 2010. HSUV for induction, consolidation, chemoresistance (CR), relapse, stem cell therapy (SCT) treatment, SCT recovery and CR post SCT were identified.

Results: Ten relevant studies were identified. Six were cost effectiveness analyses utilizing HSUVs for calculation of Quality Adjusted Life years (QALY), one effectiveness analysis (incremental QALY). Two QOL studies reporting specific AML utilities (either collected or mapped from QLQ-C30). An additional study reported QOL for patients undergoing SCT. Since no study reported HSUV for relapse, values from study of secondary AML patients who failed prior treatment for Myelodysplastic Syndrome, were used. Where multiple HSUVs were available, prioritized clinical trial (n=488). Median OS at 1-year was identified if HSUV were presented in Figure. AML treatment (both induction, consolidation and SCT) was associated with decreased HSUV, while post-treatment CR lead to increased HSUV.
E946

ITALIAN REAL LIFE EXPERIENCE OF DECITABINE IN ELDERLY ACUTE MYELOID LEUKEMIA PATIENTS: INTERIM ANALYSIS OF MULTICENTRIC OBSERVATIONAL DEA65 STUDY.


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Background: Acute Myeloid Leukemia (AML) has a higher incidence among the elderly population. Older patients (pts) with AML have a worse prognosis and limited treatment options. Hypomethylation agent decitabine was recently approved by FDA and EMEA as first line treatment in AML pts older than 65 yrs and unfit to receive standard cytotoxic chemotherapy. Decitabine showed to be superior to supportive care or low dose cytaraebine in controlled randomized clinical studies (Kantarjian, JCO 2012; Cashen, JCO 2010).

Aims: In July 2016 we approved a retrospective and prospective multicentric observational real life study to investigate efficacy and tolerability of decitabine at the approved schedule of 20mg/m² daily for 5 days of a 4-week cycle in real life (DEA65 study). The primary objective was the assessment of overall survival (OS). Secondary objectives were evaluation of adverse events (AEs) and response rate: complete remission (CR), CR with incomplete platelets or white blood cells (WBCs) count recovery (CRi), partial remission (PR) and hematological improvement with transfusion independence. We here present an interim analysis of the first 56 pts enrolled.

Methods: AML pts older than 65 yrs treated in first line with decitabine were enrolled in the study. At diagnosis and during follow-up, cytogenetic and molecular assessment was performed by each center according to local guidelines for AML management in elderly pts.

Results: Biologic and clinical data of 56 pts, with a median age of 73 yrs (range 65-90 yrs) are reported. Thirty-one patients (55,3%) had a secondary AML and 13/31 (42%) were progressed MDS previously treated with 5-azacitidine. Median WBCs count was 3050/µL (range 770-131500/µL) with 13/56 (23%) pts with WBCs<100000/µL. Cytogenetic analysis was performed in 52/56 pts, and in 24/56 (43%) molecular analysis including FLT3 and NPM1 mutations was performed. According to prognostic classification, 50% of pts had a high risk, 34% an intermediate-risk, 9% a low risk AML and in 4/56 (7%) pts risk was unknown. Median OS was 7 months (range 1-19 months) with 34/56 deaths (60,7%) and a median of 6 cycles (range 1 to 19) of decitabine. Overall response rate was 60,7% (34/56 pts), of which 7/56 (12,5%) CR or CRi; 17/56 (30,4%) PR and 10/56 (17,8%) partial remission (PR) at a certain dose level. If ≥3 of 9 patients experienced DLT, the trial was to be terminated. To evaluate Gln reduction ability of Erwinaze, the dose could be increased based on 48h trough plasma Gln in cohorts of 3, 6, or 9 pts per dose level.}

Summary/Conclusions: This interim analysis of the use of decitabine in real life showed a superimposable OS to controlled international clinical trials. Safety profile was acceptable considering setting of pts and incidence of important comorbidities. Despite a similar OS, the comparison between our data and Cashen study (56 vs 55 pts) showed in our cohort, a poorer rate of CR+CRi (60%, vs 70%) and a lower impact of FAB in elderly pts treated with 5-azacitidine therapy. WBC >10000/µL as well as high cytogenetic risk. This apparent contradiction supports the idea that in elderly pts recovery of peripheral blood counts (PR+hematological improvement) is probably the most important factor influencing OS.

E947

ASPARAGINASE ERWINIA CHRYSANTHEMI EFFECTIVELY DEPLETES PLASMA GLUTAMINE, HAS CLINICAL ACTIVITY, AND IS WELL TOLERATED IN ADULT PATIENTS WITH RELAPSED OR REFRACTORY ACUTE MYELOID LEUKEMIA

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Background: Asparaginase-induced glutamine (Gln) depletion demonstrates anti-leukemic activity in preclinical studies of AML. We hypothesized that administration of asparaginase Erwinia chrysanthemi (Erwinaze) would lead to effective plasma Gln reduction and may be a feasible therapeutic approach for AML, because myeloblasts may be addicted to Gln.

Aims: The primary aim was to determine the dose of Erwinaze inducing plasma Gln levels ≤120μmol/L, with an acceptable safety profile, 48 hours (h) after the first intravenous (IV) dose and before each subsequent dose administered thrice weekly for 2 weeks in patients (pts) with relapsed or refractory (R/R) AML. Methods: This was a phase 1, single-arm, pharmacokinetic investigator-initiated trial (NCT02283190, funded by Jazz Pharmaceuticals), with a 3+3 design with dose de-escalation/escalation rules that incorporate both safety and biochemical activity (nadir plasma Gln levels) of Erwinaze. There was no intrapatient dose adjustment. For safety, a 3rd cohort of three pts was to be added if 2 of 6 pts in the 1st and 2nd cohorts experience a dose limiting toxicity (DLT) at a certain dose level. If ≤3 of 9 patients experienced DLT, the trial was to be terminated. To evaluate Gln reduction ability of Erwinaze, the dose could be increased based on 48h trough plasma Gln in cohorts of 3, 6, or 9 pts per dose level. Correlative studies measured plasma Gln, glutamate (Glu) and asparagine (Asn) levels, plasma asparaginase activity and plasma and urine 2-hydroxylglutarate (2-HG) levels.

Results: Five pts were enrolled on study. Enrollment was then halted due to Erwinaze supply manufacturing complexities. Median age was 69 (range 20-83) years, 4 were male, 2 had prior MDS or CMLM, 3 had high risk abnormal karyotype, 3 had isocitrate dehydrogenase (2 IDH1, 1 IDH2) mutations, and 3 had been treated with ≥2 lines of prior treatment. Erwinaze was administered IV (25,000 μU/mL, dose level 0) for 6 doses MWF for 2 weeks to all pts. No DLT was observed. Anemia and electrolyte abnormalities were the most common adverse events. Plasma asparaginase activity >0.1 IU/mL was achieved in all pts at 48h trough, but in 3 pts it decreased to zero on day 8 (72h trough). Median trough plasma Gln, Asn and peak Glu levels (μmol/l) at 48h were 27.6 (range <12.5-227), 0 (range 0-0), and 704 (range 474-754), respectively. Asn remained undetectable for the entire 2 weeks. Gln levels increased significantly on day 8 (72h trough) compared to day 5, p<0.001. Four of 5 pts (80%, lower limit of 1-sided 95% CI: 34%) achieved at least one nadir Gln value <120 μmol/L. The fold reduction (FR) in Gln level at 3 days, relative to baseline, was 0.16 (p=0.051 for rejecting FR=1). One achieved partial remission (PR) and one achieved hematologic improvement (HI) after 6 doses of single agent Erwinaze. Both pts had plasma Gln levels <65 μmol/L on days 5, 10 and 12. Off study, after completion of Erwinaze, they have been treated with azacitidine. Both pts are still alive in complete remission (CR) and CR with incomplete count recovery (CRi) 13.3 and 13.4 months after the on-study date. Plasma and urine 2-HG levels did not change significantly. The 3 pts with IDH mutations tended to have higher plasma 2-HG levels (p=0.10).

Summary/Conclusions: This interim analysis of the use of decitabine in real life showed a superimposable OS to controlled international clinical trials. Safety profile was acceptable considering setting of pts and incidence of important comorbidities. Despite a similar OS, the comparison between our data and Cashen study (56 vs 55 pts) showed in our cohort, a poorer rate of CR+CRi (60%, vs 70%) and a lower impact of FAB in elderly pts treated with 5-azacitidine therapy. WBC >10000/µL as well as high cytogenetic risk. This apparent contradiction supports the idea that in elderly pts recovery of peripheral blood counts (PR+hematological improvement) is probably the most important factor influencing OS.

Table 1.
Summary/Conclusions: To the best of our knowledge, this is the first clinical report demonstrating that an aspiraginase product is capable of not only decreasing plasma Gln level to ≤120μmol/L but also depleting it to undetectable (i.e. <12.5μmol/L) levels in pts with AML. Two of 5 patients with R/R AML had clinical responses and are alive in remission. Given clinical activity of aspiraginase in AML, we are to investigate mechanistically-designed aspiraginase combination therapies.

E948
PROGNOSTIC SIGNIFICANCE OF SOX2, SOX3, SOX11, SOX14 AND SOX18 GENE EXPRESSION IN DE NOVO ACUTE MYELOID LEUKEMIA (AML) PATIENTS
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Background: Members of the SOX (SRY-related high mobility group (HMG) box) gene family encode a group of transcriptional factors with important functions in embryonic development. Also, SOX genes are aberrantly expressed in different types of cancer. However, their role in hematological malignancies, especially in acute myeloid leukemia (AML), remains elusive.

Aims: The aim of this study was to investigate the expression level of SOX2, SOX3, SOX11, SOX14 and SOX18 genes in de novo AML patients, and to evaluate their potential as prognostic markers.

Methods: Fresh bone marrow (BM) samples were collected from 50 non-APL AML patients at diagnosis (27 male, 23 female, median age 52.5 years, range 22-73) and from 8 healthy donors. Relative quantification analysis of SOX genes expression level was performed by RQ-PCR methodology, with GAPDH gene as endogenous control, and using comparative ddCt method with healthy controls as calibrator.

Results: The median expression level of SOX2, SOX3, SOX11, SOX14 and SOX18 in AML patients was 0.46 (0.01-226.13), 0.81 (0.01-1210.00), 0.35 (0.01-177.29), 0.98 (0.02-469.51) and 3.53 (0.18-332.00), respectively. This was not significantly different from the levels detected in healthy controls where the median expression levels were 1.00 (0.32-2.54), 1.00 (0.45-5.73), 1.00 (0.19-12.57), 1.04 (0.38-2.38) and 1.00 (0.48-12.29), respectively. As a cut-off value above which the patients were considered to be positive for SOX2/3/11/14/18 gene expression we used median expression level of each SOX gene in healthy controls + 2SD. The percentage of patients who were positive for the expression of the studied genes ranged from 14% (SOX2* and SOX11*), 20% (SOX3* and SOX18*) to 28% (SOX14*). A significant association with the presence of FLT3-ITD and NPM1 mutations was detected in all but SOX14+ patients. The same result was found concerning association with higher leukocyte count. There were no significant associations with any other presenting clinical parameters. As for the impact that SOX gene expression positive status had on the analyzed genes had on the prognosis and outcome of the disease, we detected higher relapse rate in SOX14+ patients (p=0.045). Significantly shorter disease-free-survival (DFS) was detected among SOX2*, SOX11* and SOX18* patients (p<0.001; p=0.001; p=0.017, respectively). Although all of the patients, the most prominent influence has been detected for the SOX2* patients, the most prominent influence has been detected for the SOX2* patients (p=0.034).

Summary/Conclusions: This is the first study focused on examining the expression level of SOX2/3/11/14/18 in AML patients. We have found that these genes are overexpressed among patients in comparison with normal BM. However, in some patients, the expression of these genes is highly increased, and associated with a negative prognostic factors such as the presence of FLT3-ITD mutations and higher leukocyte count. Also, increased expression of these genes has been clearly associated with shorter DFS and OS. This indicates that the exact function of these genes in the pathogenesis of AML is not yet known, our preliminary results show that their overexpression can have prominent prognostic significance in AML patients and therefore should be the subject of further investigation.

E949
ACUTE ANTHRACYCLINE INDUCED CARDIOTOXICITY IN PATIENTS WITH ACUTE MYELOID LEUKEMIA
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Background: Chemotherapeutic agents are associated with a wide range of cardiotoxic adverse effects. Anthracyclines and related drugs are some of the most implicated agents, with a well-recognized potential for the development of cardiomyopathy and heart failure. Chronic anthracycline induced cardiotoxicity can lead to cardiomyopathy, which may develop several years after treatment. Acute and subacute anthracycline induced cardiotoxicity is considered relatively uncommon, described mostly in patients treated for solid tumors or lymphomas. While anthracycline based regimens have been used to induce remission in newly diagnosed patients with acute myeloid leukemia (AML) for more than four decades, relatively little is known about the acute cardiotoxic effect of anthracyclines in this setting. Since many of these patients were candidates for hematopoietic stem cell transplantation (HSCT), an intensive intervention usually reserved for fit patients, even transient decrease in cardiac function might render them ineligible for this intervention, or might increase their transplant related morbidity.

Aims: To study the short-term outcomes of anthracycline exposure on cardiac function in patients with AML who are candidates for allogeneic HSCT. Because current AML-induction regimens use anthracyclines (most commonly daunorubicin) at a relatively high dose between 45 and 90mg/m2/day for three consecutive days, we hypothesized that the incidence of post-induction cardiac injury in these patients would be high.

Methods: The medical records of 55 consecutive patients who had received induction chemotherapy and had undergone HSCT in our medical center were reviewed. Patients included in the study were those with echocardiographic data both prior to and post induction therapy. Median age at diagnosis was 59 years (range: 19-73) and 49% were males. Approximately half of the patients had de novo AML (N=29, 53%), 26 patients (47%) had either therapy related AML or AML secondary to a previous hematological disorder. Induction treatment included 7 days of cytarabine at a dose of 100mg/m2/day and 3 days of daunorubicin at a dose of 45mg/m2/day (N=2, 3.6%), 60mg/m2/day (N=34, 61.8%) or 75mg/m2/day (N=15, 27.3%).

Results: Selected patient characteristics are summarized in Table1. Post-induction echocardiograms demonstrated a significant cardiac deterioration in left ventricular ejection fraction (EF) (defined as 10% or more absolute decrease from baseline EF) in 25.5% of the patients (N=14). Higher doses (90mg/m2/day) of anthracyclines were associated with a higher risk of cardiac function deterioration (odds ratio: 4.1. 95%, confidence Interval: 1.06 to 15.7). Patients with cardiovascular risk factors and male patients tended to develop cardiotoxicity at higher rates, whereas age, white blood cell counts at diagnosis and AML type (de novo vs. secondary) had no impact on cardiotoxicity. The decrease in cardiac function was temporary in 10.9% of the patients (N=6) with subsequent normalization of left ventricular EF in those patients.

Summary/Conclusions: The use of daunorubicin at a dose of 60mg/m2/day or less is associated with significantly lower rates of acute cardiotoxicity. Our findings should be taken into consideration when choosing the anthracycline dose, particularly in male patients with cardiovascular risk factors who are candidates for HSCT.
AN INTEGER WEIGHTED GENOMIC MUTATION SCORING (IWGMS) USING THE TRUSIGHT MYELOID SEQUENCING PANEL SHOWS HIGHER MORTALITY IN PATIENTS WITH INTERMEDIATE RISK ACUTE MYELOID LEUKAEMIA - A RETROSPECTIVE STUDY

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Background: AML is currently classified by European LeukemiaNet into favorable, unfavorable, and intermediate prognosis based on cytogenetic aberrations. Although favorable and unfavorable categories have good prognostic values, the intermediate category encompasses the majority of patients and offers unclear prognosis. The development of Cancer Genome Atlas (TCGA) opens new windows for the incorporation of next generation sequencing (NGS) into cytogenetics to enhance prognostic risk stratification. However, few studies explore the combination of cytogenetics and NGS in prognostic predictions.

Aims: Here we have developed a system of Integer Weights for the Genomic Mutation Score (IWGMS) for a quantifiable stratification of the prognostic risks associated with a combination of cytogenetic aberrations and genomic mutations. Our next step is validating the scoring system through its application to data obtained from other institutions.

Methods: Patient data at Houston Methodist Hospital was queried from Methodist Environment for Translational Enhancement and Outcomes Research (METEOR), a clinical data warehouse that integrates research databases and national registries. The diagnosis of AML was queried along with patient demographics, cytogenetics, NGS and OS. The resultant patients were divided into three categories based on their MRC cytogenetic risks: favorable, intermediate, and poor. Using the TruSight Myeloid Sequencing Panel (illumina), mutations in 54 genes associated with myeloid disorders were tested in NGS. A scoring system that xenogen each of the nine TCGA mutation categories (Transcription- Factor Fusion, Nucelophostin (NPM1), Tumor Suppressor Genes, DNA-Methylation related genes, Signaling Genes, Chromatin Modifying Genes, Myeloid Transcription Factor Genes, Cohesion complex Genes and Spliceosome-complex genes) a score between -2 (good risk) and +2 (poor risk). The IWGMS for each patient was calculated by the sum of the individual mutation scores. A IWGMS greater than 3 was considered significant as a poor prognostic factor. Statistical analysis was done using Chi-Square, Mann Whitney U test and multivariate logistic regression analysis. Data from other institutions will be analyzed in a similar fashion for the confirmatory portion of the project.

Results: A hundred of the 1200 AML patients met the criteria for having both cytogenetic and NGS data availability. The two-year mortality rates were 43%, 52%, and 51% respectively for the favorable, intermediate, and poor cytogenetic groups. In the intermediate cytogenetic group, high IWGMS score (>3) was associated with higher mortality when compared to low IWGMS score (80% vs 44%, p=0.045, Fig 1). A look at the gene mutation distribution in the intermediate risk cytogenetic group also showed a general correlation between known favorable gene mutations with low IWGMS scores and unfavorable ones with high IWGMS scores. We thus hypothesize the IWGMS scoring system can be utilized to divide intermediate cytogenetic and low and intermediate mortality subgroups based on a combination of cytogenetic and genetic mutations. We expect similar results with data from other institutions.

Summary/Conclusions: Most studies in current literature focuses on the individual contributions of cytogenetic aberrations or genetic mutations to risk stratification and treatments risk stratification and treatment response. However, prognosis varies widely in the heterogeneous, intermediate cytogenetic class, where 60% of the AML patients belongs. We propose a systematic approach that integrates cytogenetics with genetic mutations in stratifying prognostic outcomes with a focus on the intermediate cytogenetics group. The ability to differentiate in this specific group opens great potentials for targeted therapies and improving outcomes.
Aims: Since the roles of these SNPs in clinical aspects, response to therapy and prognosis of DLBCL treated with R-CHOP are still unknown, these were the aims of the present study.

Methods: Our analysis included 168 consecutive DLBCL patients at diagnosis seen at University Hospital from July 2009 to September 2014. Genotypes were identified in DNA of peripheral blood by real-time polymerase chain reaction using a Taqman SNP genotyping assay. Replicates were performed in 10% of the reactions, achieving 100% of concordance. Chi-Square test, Fisher’s Exact test, and multivariate analysis, using the logistic regression model, served to assess associations between genotypes and clinical aspects. Kaplan-Meier analysis was used to evaluate the effect of clinical features and genotypes on progression-free survival (PFS) and overall survival (OS). EFS and OS were calculated from the date of diagnosis to first event date (relapse, progression or death by disease) or last seen date and death by any cause or last seen date, respectively. Cox proportional hazards regression model was used to evaluate the effects of clinical features and genotypes of the above mentioned SNPs on PFS and OS, and the results of analysis were presented as hazard ratios (HRs) with their corresponding 95% confidence intervals (CIs). First, these associations were examined using univariate Cox proportional hazards regression. In a second step, all variables with P<0.10 were included in a multivariate Cox regression. All reported P values were two-sided, and P<0.05 was considered to indicate statistical significance.

Results: Concerning clinical features, the frequency of the wild-types VEGF -1154G allele and VEGF -634G genotype were more common in stage II or IV patients. The wild-type VEGFR2 -604TT genotype was more common in high intermediate and high international prognostic index (IPI) patients. Concerning response rate, patients with the wild-type VEGF 936CC genotype was associated with higher complete response (CR). These patients had 2.65 more chances of achieving CR to therapy than others. The median follow-up time of 168 DLBCL patients enrolled in the study was 43 months (range: 1-105). The estimated probabilities of 60-months EFS and OS were 58.8% and 66.0%, respectively. At 60 months of follow-up, patients with the variant VEGF 1154 A and 936 T alleles had 1.52 and 1.52 more chances of presenting relapse disease or progression, and 1.47 and 1.60 more chances of evolving to death in univariate analysis, respectively. After correction with other classical prognostic factors in DLBCL (IPI and GCB subtype), only the VEGF 1154 G/A SNP was associated with PFS and OS: patients with the variant VEGF 1154 A allele had 1.88 and 1.83 more chances of having an event.

Summary/Conclusions: Our data present, for the first time, preliminary evidence that inherited abnormalities in AG pathway, related to the VEGF -1154G/A, -634GG and 936CC/T, influence clinical features, leading to a change in the risk of patients with a worse survival. In this cohort, BMI by PET/CT could not independently predict a shorter PFS and/or OS.

E954
THE PROGNOSTIC SIGNIFICANCE OF CD11B+CX3CR1+ MONOCYTES IN PATIENTS WITH NEWLY DIAGNOSED DIFFUSE LARGE B-CELL LYMPHOMA
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Background: Interest in the role of myeloid-lineage cells, including monocytes and their precursors, has been increasing in prognosis of lymphoma. It has been shown that the circulating monocyte count at the time of diagnosis shows prognostic significance in diffuse large B-cell lymphoma (DLBCL), suggesting the role of specific subset of monocyte in prognosis of DLBCL. Recent studies suggest CD11b+ monocytes expressing CX3CR1 promote angiogenesis and suppress anti-tumor immunity through the interaction with fractalkine (CX3CL1), the only ligand for CX3CR1. However, limited data is available regarding the prognostic significance of CD11b+CX3CR1+ monocytes in DLBCL patients.

Aims: The study investigates the prognostic significance of peripheral blood (PB)- and bone marrow (BM)- CD11b+CX3CR1+ monocytes on progression-free survival (PFS) and overall survival (OS) in newly diagnosed DLBCL patients treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) therapy.

Methods: The prospective study was conducted in two Korean institutions from May 2011 to August 2015. Patients were eligible if they were newly diagnosed DLBCL, treated with R-CHOP, and provided informed consents. Percentages of CD11b+CX3CR1+ cells in total mononuclear cells (>50,000 cells) were measured by flow cytometric analysis using fresh PB and BM aspirates before treatment.

Results: Eighty-nine patients (male, 52) were enrolled. The median age was 65 years (range, 19-88). 37 patients (41.6%) were classified as high-intermediate (III) or high risk according to International Prognostic Index (NCCN-IPI). CD11b+CX3CR1+ monocytes were significantly higher in PFS and OS analysis. The expression percentage of CD11b+CX3CR1+ cells was 3.31% (range, 0.21 to 21.66%) in PB and 3.09% (range, 0.20-20.01%) in BM. Patients were categorized into high (PB- or BM-CD11b+CX3CR1+ cells >median) and low (<median) groups. High PB-CD11b+CX3CR1+ cell group was significantly associated with unfavorable clinical outcomes, including age >60 years, advanced stage, elevated serum lactate dehydrogenase level, and extranodal involvement (P<0.05), which were clinical factors associated with higher risk NCCN-IPI (P=0.004). However, BM-CD11b+CX3CR1+ cells were not associated with clinical variables. With a median follow-up of...
27.7 months (IQR, 14.6-48.1), low PB-CD11b+CX3CR1+ cell group had significantly better PFS (3-year, 77.1% vs 58.7%; P=0.006) and OS (3-year, 86.6% vs 58.4%; P=0.004) than high PB group. No significant survival differences were observed between high and low BM-CD11b+CX3CR1+ cell groups. Uni-variate analyses demonstrated that age, ECOC performance status, B symptoms, extranodal involvement, NCCN-IPI, and PB-CD11b+CX3CR1+ cell group were significantly associated with OS. However, HI or high risk NCCN-IPI was an only independent prognostic factor for reduced OS (hazard ratio, 4.41; 95% confidence interval, 1.17-16.59) in the multivariate analysis. In subgroup analysis according to the NCCN-IPI, 3-year OS of high PB-CD11b+CX3CR1+ monocytes was significantly inferior to that of low group (34.0% vs 77.9%; P=0.026) in B-NHL, while no high risk NCCN-IPI-related monocytes failed to predict OS (3-year, 91.7% vs 96.7%; P=0.878) in the low to low-intermediate risk NCCN-IPI subgroup.

Summary/Conclusions: Our study represents PB-CD11b+CX3CR1+ monocytes can be utilized in differential patients with high risk for early death and are associated with risk stratification by the NCCN-IPI, possibility of potential therapeutic target in DLBCL.

E955

RARE NON-HODGKIN LYMPHOMAS (R-NHLs) IN CHILDREN: THE AIEOP EXPERIENCE

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Background: Clinical management of pediatric rare non-Hodgkin lymphomas (r-NHL; i.e., one/two cases) is unclear.

Aims: To characterize children with r-NHLs in AIEOP centers. Performing a retrospective analysis of r-NHLs AIEOP case records, describing the main epidemiological, clinical and pathological parameters. To review the histopathological case records according to WHO 2008 classification. Evaluation of treatment response - chemotherapy or wait and see (W&S) in terms of overall survival (OS) and of complete remission (CR), relapse and resistance cases, secondary neoplasms and deaths.

Methods: Data from the AIEOP database were collected between 1997 and 2015. Results: The incidence of r-NHL in AIEOP registry was 6.5% (67 pts). Forty-eight were male and 19 female, median age was 11 years (0-3-21 years). Classification according to St.Jude stage was: stage I n=36; II n=13; III n=11; IV n=7. Bone marrow (BM) involvement was diagnosed in 7 cases; central nervous system (CNS) in one case. Patients who presented LDH >500 UI were 18. B-NHLs accounted for approximately 49% (33 pts) of the entire population analysed. NHL consisted of 74% (27 pts), the remaining 11% (7 pts) of the population under study being categorized as “others” (other than those deriving from B or T/NK-cells). The most common histological subtypes were: follicular lymphoma (FL) amongst B-NHLs; peripheral T-cell lymphoma (PTCL) n.o.s., mycosis fungoides (MF), subcutaneous panniculitis T-cell lymphoma (SPTCL) and lymphomatoid papulosis (LP) amongst T-NHLs; histiocytic sarcoma (HS) amongst “others” category. A similar proportion for both B and T/NK NHL underwent either W&S approach only or active treatment (AT): 45% and 55% were W&S and AT approach, respectively. Patients in “others” category were almost actively treated (71%). Therapy was based on AIEOP B-, T/NK-NHLs and ALC1 protocols, CHOP, and/or immunotherapy. Surgical resection has been performed in case of localized disease B-NHLs only, followed by a W&S strategy, with 100% 3-yr OS. It has been seen that B-NHLs have a more favorable prognosis and very few events (development of resistance to therapy, relapse, secondary malignancy, death). Amongst T/NK NHLs-related events, death remained the most common outcome: 21/56 cases (37.5%) died, whereas 17/110 cases (15.5%) underwent a slight number of relapses; as for the category “others”, no relative preponderance has been registered for any of the above-mentioned events. The 3-year OS has shown to be significantly higher for B-NHLs than for T/NK-NHL (94% vs 69%, p-value 0.024), as illustrated in Figure 1. Regarding the treatment, the 3-year OS was 100% for the patients underwent a W&S approach whereas 75% for treated patients (p-value 0.037). FLs show favourable clinical course and outcome, limited stage at diagnosis. Differently from adults, pFLs have a higher 3-years OS with respect to that of other histological pediatric NHLs subtypes (100% vs 75%, p-value 0.049).

Figure 1. Summary/Conclusions: The incidence of AIEOP pediatric r-NHLs is in line with the literature. In case of localized disease, a W&S approach was successfully applied; of these, the T/NK NHLs being most often registered and with best prognosis are the cutaneous lymphomas (i.e. LyP, MF). Patients’ prognosis varies greatly depending on the histological subtype. The better survival was observed in the B-NHLs compared to other categories. An international collaboration is warranted, in order to create new guidelines or protocols for an appropriate management of pediatric r-NHLs.

E956

PRIMARY ANALYSIS OF THE EFFECT OF HEMATOPOIETIC STEM CELL TRANSPLANTATION IN THE TREATMENT OF 110 CASES OF T CELL LYMPHOMA

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Background: T cell lymphoma(T-NHL) is a rare and heterogeneous group of lymphoid malignancies with mostly poor outcome with conventional treatment. Recent studies have suggested that Hematopoietic stem cell transplantation(HSCT) has a better curative effect and is superior to traditional chemotherapy.

Aims: To investigate the effect of HSCT in the treatment of T cell lymphoma. Methods: The clinical data of 110 patients with T cell lymphoma treated by HSCT from January 2006 to August 2016 in our center were retrospectively analyzed.

Results: (1)110 T-NHL patients, 70 males and 40 females, aged 7-64 years (median age 26 years). Disease subtypes: 35 cases of T-cell lymphoblastic lymphoma(T-ALL), 23 cases of NK / T cell lymphoma(NK/TCL), 24 cases of peripheral T-cell lymphoma (PTCL, NOS), 24 cases of variable large cell lymphoma (ALCL), 3 cases of subcutaneous panniculitic T cell lymphoma(SPTCL) and 1 case of hepatosplenic T cell lymphoma(HSTCL). Transplantation type: 56 cases of autologous hematopoietic stem cell transplantation (auto-HSCT), 54 cases of allogeneic hematopoietic stem cell transplantation (allo-HSCT). The follow-up was ended in December 2016, the duration of following-up ranged from 2 to 130 months (median follow-up time was 22 months). (2)36/110 patients with auto-HSCT, 3 year overall survival (OS) and disease-free survival (DFS) were 76.5% and 60.9%, respectively. (3)54/110 patients with allo-HSCT, 3 year OS and DFS of allo-HSCT were 61.7% and 58.9%, respectively. (4)36/56 patients with CR1 status before auto-HSCT, 3 year OS and DFS were 60.6% and 40.2%, respectively. The OS and DFS of the two groups were significantly different (P=0.001). (5)45/110 cases were young and high-risk patients (age<60 years, IPI score >3).25/54 cases treated with allo-HSCT, the 3 year OS and DFS were 62.8% and 60.8%, respectively. 20/56 patients with non-CR1 status before auto-HSCT, 3 year OS and DFS were 47.6% and 36.9%. The OS and DFS of the two groups were also significantly different (P=0.001).

Summary/Conclusions: HSCT can improve the efficacy of T cell lymphoma. Auto-HSCT in first complete remission (CR1) enables T-NHL patients with
greater benefit. Alto-HSCT can cure some T-NHL patients, which can be considered for the treatment of young and high-risk T-NHL patients.

**E957**

**SHORT COURSE OF R-HYPERCVAD/MITX/ARA-C FOLLOWED BY ASCT AS FIRST-LINE THERAPY IN MANTLE CELL LYMPHOMA PATIENTS PROLONGS PROGRESSION FREE SURVIVAL TO MORE THAN 9 YEARS.**

**SINGLE CENTER EXPERIENCE**

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**Background:** Mantle cell lymphoma (MCL) is considered an incurable disease with an historical median overall survival around 3-4 years with short progression free survival (PFS) periods. Regimens that include high dose cytarabine and consolidation with autologous stem cell transplant (ASCT) have become standard therapy for fit patients. The median PFS reported after 4-6 cycles HyperCVAD followed by ASCT consolidation is 4.5 years (Ahmadi et al, BMT 2012). Nevertheless, toxicity is high and many patients cannot obtain stem cells for transplant. In this setting, some groups use 6-8 cycles R-HyperCVAD without ASCT consolidation, achieving the same median PFS of 4.6 years (Romaguera et al, Br J Hematol 2010). Based on this we have reviewed our experience using a short course of HyperCVAD followed by transplant consolidation.

**Aims:** To analyze our experience treating fit patients with MCL in first line with a short course of 2 cycles of R-HyperCVAD followed by consolidation with ASCT.

**Methods:** From January 2002 to August 2016, the patients diagnosed with MCL treated in first line with a short course of 2 cycles of R-HyperCVAD and ASCT were included in this retrospective analysis. International working group response assessment criteria were used, PFS was calculated from the date of start therapy until date of relapse/progression or last contact.

**Results:** During the study period 85 MCL patients were registered: 7 (8.2%) did not receive immediate therapy, 44 (52.4%) were not eligible for intensive therapy due to comorbidities or age and 33 (39.3%) were treated in first line with a short course of 2 cycles of R-HyperCVAD and ASCT. Clinical characteristics at diagnosis of these 33 patients were: M/F ratio: 26:7 (78.8%-21.2%), median age: 63 y.o (limits: 40-73), ECOG 0-1: 26 (86.7%), Ann Arbor stage III-IV 28/31 (90.3%), MIPI score: low risk: 5 (16.7%), intermediate risk: 17 (56.7%), high risk: 8 (26.7%). Thirty (90.9%) patients completed the 2 cycles of R-HyperCVAD. Reasons for discontinuation were: 2 deaths for sepsis and 1 CNS progression. Intention to treat response rate was: CR 26 (78.8%), PR 2 (6.0%), progressive disease 3 (9.0%), not evaluable 2 (6.0%). Among the 28 patients in CR / PR considered eligible for consolidation with ASCT, 8 patients were not transplanted: 4 (14.3%) had harvest failure (all before plerixafor availability), 2 had persistent toxicity (prolonged neutropenia and severe mucositis) and were not considered for ASCT. 1 rejected, 1 unknown cause. Conditioning regimen was BEAM/LACE in 18 (90%) patients and cyclophosphamide-TBI in 2 (10%). One patient died 10 days after infusion for sepsis. With a median follow-up of 35 months (1-131 months), the median PFS was 73.0 (90% CI: 38.2-107.8) months (8.08 years) for the whole group, 114 (47.3-180.7) months (9.4 years) for the transplanted patients vs 21 (3.1-38.9) months (1.8 years) for the not transplanted group. The median OS was 123 (31.9-214.1) months, median OS was not reached for transplanted group vs 31.0 (7.5-54.6) months for not transplanted.

**Summary/Conclusions:** A short course of R-HyperCVAD achieves a very high remission rate in fit patients with MCL. Stem cells could not be obtained in a small proportion of patients, all of them before the use of plerixafor. Two thirds of the patients could complete the planned therapy with ASCT consolidation, and those patients have an excellent outcome, with a PFS of more than 9 years.

**E958**

**THE FREQUENCY OF INCIDENTAL MALIGNANCIES DETECTED BY PET/CT SCANS IN PATIENTS WITH LYMPHOMA AND THE ASSOCIATED CLINICAL IMPLICATIONS**

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**Background:** PET/CT imaging has a well-established role in the investigation of malignant lymphoma. Given the widespread clinical applications, unexpected findings are occasionally identified. Whilst there is substantial information pertaining to additional primary cancers identified on PET/CT in patients with solid organ malignancy, there is a relative paucity of data in patients with lymphoma.

**Aims:** The primary aim was to identify the frequency of incidental second malignancies detected by PET/CT imaging in patients with lymphoma. Qualitative data related to histological diagnosis and staging, interruptions or obstacles to lymphoma therapy, therapy for the second malignancy and the overall impact upon prognosis were also reviewed.

**Methods:** A total of 550 PET/CT images were performed in 298 patients at The Prince of Wales Hospital, Sydney Australia between January 2013 – March 2016. Patients with both Hodgkin’s and Non-Hodgkin’s lymphoma, with PET/CT imaging performed for all medicare-approved indications were included. All PET/CT reports suggested of an incidental second malignancy prompted further review of electronic medical records, MOSAIC cancer database and paper medical records. Where a clear diagnosis of second malignancy was confirmed, information regarding histological findings and staging, as well as the implications of this diagnosis related to treatment of the underlying lymphoma and impact on overall prognosis was collected.

**Results:** 510 PET/CT scans in 259 patients had confirmed diagnoses of lymphoma. Patients aged 17 to 96 were included in the study, with a median age of 62 years. Of the 259 patients included (M=155; F =104), 55 patients had a diagnosis of Hodgkin’s lymphoma and 204 patients a diagnosis of Non-Hodgkin’s lymphoma. A total of 33 out of 259 patients with a diagnosis of malignant lymphoma had PET/CT findings suspicious for an underlying second malignancy (12.7%). Of the 33 patients, 19 underwent further invasive investigation, with a total of 8 patients having a biopsy proven histological diagnosis of a second malignancy (3.1%). Qualitative information was gathered regarding the patients who did not have further investigation.

**Summary/Conclusions:** The frequency of incidental malignancies detected by PET/CT imaging in patients with lymphoma was found to be comparable to other similar international retrospective studies. The majority of incidental second malignancies were early stage and gastrointestinal in origin. Further retrospective as well as prospective data may assist in the establishment of guidelines, to address a standardized diagnostic approach to investigating incidental lesions discovered on PET/CT imaging that are suggestive of a second malignancy.

**E959**

**CLINICAL IMPACT OF KARYOTYPIC EVOLUTION ON THE PROGNOSIS OF DIFFUSE LARGE B CELL LYMPHOMA**

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**Background:** We retrospectively examined the medical records of 465 DLBCL patients who were diagnosed and treated with either rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP) or with a R-CHOP-like regimen at three independent institutes in Kyoto, Japan, between January 2006 and April 2014. We analyzed the relationship between the number of subclones and prognosis utilizing the Kaplan-Meier curve and Cox proportional hazards regression analysis. We also utilized Fisher’s exact test to investigate the correlation between the number of subclones and the conventional prognostic indexes, i.e. R-CHOP, NCCN-CHOP, and KPI. This study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

**Aims:** We investigated the clinical impact of karyotypic evolution on the treatment outcome of DLBCL.

**Methods:** We retrospectively analyzed the medical records of 465 DLBCL patients who were diagnosed and treated with either rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP) or with a R-CHOP-like regimen at three independent institutes in Kyoto, Japan, between January 2006 and April 2014. We analyzed the relationship between the number of subclones and prognosis utilizing the Kaplan-Meier curve and Cox proportional hazards regression analysis. We also utilized Fisher’s exact test to investigate the correlation between the number of subclones and the conventional prognostic indexes, i.e. R-CHOP, NCCN-CHOP, and KPI. This study was conducted in accordance with the ethical principles of the Declaration of Helsinki.
and was approved by the institutional review boards of all participating institutes.

**Results:** Among the 465 DLBCL cases, karyotypic analyses by G-banding were performed on biopsied tumor specimens before the start of treatment in 181 patients. Among the 181 patients, metaphase spreads were available for G-banding in 120 patients. Neither overall survival (OS) nor progression free survival (PFS) was statistically significantly different between the patients with available metaphase and no available metaphase spreads. Based on the result of G-banding, we next divided the 120 patients with available metaphase spreads into two groups, i.e., patients with karyotypic abnormalities accompanied by ≥2 subclones and patients with 0-1 subclones. We found that the presence of ≥2 subclones was significantly associated with poor OS (3 year OS rates of patients with ≥2 subclones and 0-1 subclones were 67.6% and 82.8%, respectively (p=0.035), and tended to associate with a shorter PFS. Among the 120 patients with available metaphase spreads, the R-IPI-defined high-risk patients and IPI-defined high-risk patients were significantly more frequent in the group of patients with ≥2 subclones. Ages and genders were not significantly different between patients with ≥2 and with 1-2 subclones.

**Summary/Conclusions:** DLBCL is a cytogenetically and molecularly heterogeneous disease entity. No specific chromosomal abnormality has been associated with the shorter survival, except double or triple hit lymphomas. However, in this study, it was possible to divide DLBCLs into two groups based on karyotypic evolution, i.e., DLBCLs with 0-1 subclones and with ≥2 subclones, because the OS was the most markedly different between these two groups. In our study, more subclones were associated with poor prognosis, suggesting the significance of karyotypic evolution in DLBCL. In conclusion, our study suggests that more advanced cytogenetic clonal evolution underlies the development of high-risk disease feature in DLBCL.

**E960 REGIMEN INTENSIFICATION MAY IMPROVE OUTCOMES IN PATIENTS WITH HIGHER RISK HUMAN IMMUNODEFICIENCY VIRUS (HIV) RELATED AGGRESSIVE B-CELL LYMPHOMAS**

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**Background:** Despite effective combination antiretroviral therapy for HIV, there remains an increased incidence of HIV related B-cell Non-Hodgkin lymphomas (NHL). The introduction of early antiviral therapy and effective chemotherapy have led to improved outcomes overall. Regimen intensification (RI) in HIV associated B-cell NHLs has shown improved survival, especially in the rituximab era (Barta et al, Blood 2013).

**Aims:** To examine the effect of RI on the overall survival (OS) and progression free survival (PFS) compared to CHOP based chemotherapy according standard risk stratification.

**Methods:** Patients with HIV associated aggressive B-cell NHL were identified between 2001- 2015 at Moffitt Cancer Center. Patients with primary central nervous system lymphoma, T-cell NHL and indolent NHLs were excluded. Patients received R-CHOP or intensive chemotherapy (IC) including DA-EPOCH, hyperCVAD or CODOX/IVAC as initial treatment. Data collected included patient demographics, disease baseline characteristics, CD4 count, HIV viral load, treatment regimen, response, and outcomes including relapse and OS. The IPI score was calculated, and patients were divided into two groups: lower risk group (low and intermediate IPI risk) and higher risk group (high-intermediate and high). Descriptive statistics were used for baseline characteristics. Kaplan Meier method was used to estimate PFS and OS, and the log-rank test was used to compare OS and PFS between lower and higher risk groups.

**Results:** A total of 83 patients were included. The M:F ratio was 9:4. Median age was 45 years (y) (range 25 – 65). Two thirds of patients were Caucasian. The median time from HIV to NHL diagnosis was 29 months (range 0 – 284). Eighty two percent presented with stage III/IV disease. Bulky disease was present in 27%, elevated LDH in 66%, and CD4 count<100/μl in diagnosis in 22% patients. Fifty percent of patients were on HAART therapy at time of lymphoma diagnosis (PFS) was statistically significantly different between the patients with available metaphase and no available metaphase spreads. Based on the result of G-banding, we next divided the 120 patients with available metaphase spreads into two groups, i.e., patients with karyotypic abnormalities accompanied by ≥2 subclones and patients with 0-1 subclones. We found that the presence of ≥2 subclones was significantly associated with poor OS (3 year OS rates of patients with ≥2 subclones and 0-1 subclones were 67.6% and 82.8%, respectively (p=0.035), and tended to associate with a shorter PFS. Among the 120 patients with available metaphase spreads, the R-IPI-defined high-risk patients and IPI-defined high-risk patients were significantly more frequent in the group of patients with ≥2 subclones. Ages and genders were not significantly different between patients with ≥2 and with 1-2 subclones.

**Summary/Conclusions:** DLBCL is a cytogenetically and molecularly heterogeneous disease entity. No specific chromosomal abnormality has been associated with the shorter survival, except double or triple hit lymphomas. However, in this study, it was possible to divide DLBCLs into two groups based on karyotypic evolution, i.e., DLBCLs with 0-1 subclones and with ≥2 subclones, because the OS was the most markedly different between these two groups. In our study, more subclones were associated with poor prognosis, suggesting the significance of karyotypic evolution in DLBCL. In conclusion, our study suggests that more advanced cytogenetic clonal evolution underlies the development of high-risk disease feature in DLBCL.
patients with tumor localized in extranasal sites seemed to have higher expression of BCL2 than higher DFS than nasal lesions (p=0.014 and 0.042, respectively). In univariate survival analysis, either high expression of MYC or BCL2 was significantly correlated with inferior PFS and OS (p<0.05). According to the DHS, patients with ENKTL could be divided into three significantly different risk groups for DFS and OS (3-year DFS rate for DFS of 0, 1, and 2 was 60%, 41%, and 21%, respectively, respectively, p=0.006; 3-year OS rate for DFS of 0, 1, and 2 was 79%, 49%, and 33%, respectively, p=0.015). In multivariate survival analysis, it was found that DFS was an independent prognostic factor for both PFS and OS (p=0.006 and 0.011, respectively).

Summary/Conclusions: Our study demonstrated that high expression of PD-1 on tumor cells, as well as the presence of PD-1 positive lymphocytes in the epidermis in patients with MF, may be a predictor of clinical outcome.

Table 1.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients</th>
<th>Frequency</th>
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<td>Age (years)</td>
<td>20-30</td>
<td>35</td>
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<tr>
<td>Stage</td>
<td>I-II</td>
<td>37</td>
<td>52.5</td>
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<tr>
<td>Intensity of PD-1 positive cells</td>
<td>Strong</td>
<td>45</td>
<td>67.5</td>
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<tr>
<td>Turferral and extramural PAMNS</td>
<td>Yes</td>
<td>45</td>
<td>67.5</td>
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<tr>
<td>Extramural extradermal PAMNS</td>
<td>No</td>
<td>25</td>
<td>37.5</td>
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<tr>
<td>Advanced stage of MF</td>
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<td>45</td>
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<td>Degree of epidermal infiltration</td>
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</table>

Figure 1. Kaplan-Meier survival analysis for progression-free survival (PFS) (A) in patients with MF with spontaneous regression treated with CHOP/CHOP-like rituximab. DFS is defined as the first day of chemotherapy to progression.

Figure 1. Methods: Histological preparations of 85 patients diagnosed with MF were evaluated. Survival analysis was performed with the Kaplan-Meier method. A univariate analysis was performed with clinical variables (stage and age) and anatomicopathological variables (i.e. intensity of the inflammatory inflam-
E964
CIRCULATING MICRONORTS AS BIOMARKERS IN DIFFUSE LARGE B-CELL LYMPHOMA: A PILOT PROSPECTIVE LONGITUDINAL CLINICAL STUDY
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Background: Diffuse large B-cell lymphoma (DLBCL) is highly heterogeneous in terms of phenotype and treatment response in patients. These characteristics make the prognosis difficult to establish and hinder the use of new personalized treatments in clinical practice. In this context, there is currently a necessity to define new biomarkers enabling a better definition of DLBCL subtypes, prognosis evaluation and an overview of the resistance to chemotherapeutics. We decided here to focus on circulating microRNAs that are found in all biological fluids. Their accessibility makes them good candidates for biomarkers studies.

Aims: This research aims at studying microRNAs found in plasma from DLBCL patients and at investigating their potential as biomarkers of survival in these patients. For this purpose, a plasma biobank was created with samples from DLBCL patients at different times of their treatment. This follow-up of microRNAs level during the course of treatment is particularly innovative in this study.

Methods: A plasma biobank from DLBCL patients was set up at the Centre Hospitalier Universitaire (CHU) UCL Namur Yvoir, Belgium (ethical agreement number B039201419613). Informed consents of all patients were obtained. In this way, blood samples from patients were taken before any treatment (C0), at the administration of the second and the fourth chemotherapeutic cure (C2 and C4) and at the remission review (Cf). In the case of a autograft, a sample was taken at the post-graft review (Cpg). The first step of this study was the selection of the microRNAs that will be quantified in all the samples of the biobank and that would potentially be used as biomarkers. To this end, a quantification of 377 microRNAs was performed by TaqMan® Low Density Array on the plasma samples of two selected DLBCL patients and one healthy donor with no history of cancer. These DLBCL patients were selected based on their highly different response to treatment. One of them obtained a complete remission after a CHARTx treatment, while the other presented a refractory disease to the same treatment. Thereafter, we determined some criteria to use in a scoring system to evaluate their potential as biomarkers. In this way, one point was given to a microRNA each time it meets the criteria enabling it to be defined as a potential diagnostic, prognostic and/or remission biomarker. In addition, we define a disease progression biomarker of an inherent resistance to treatment, and/or biomarker of an acquired resistance to treatment.

Results: On the 377 microRNAs quantified into the plasma of the 3 selected donors (2 DLBCL patients and 1 healthy donor), 81 microRNAs were detected. Three microRNAs obtained the highest score of 5 points: miR-122, miR-19a and miR-21. Four points were attributed to miR-122, miR-19a and miR-21. Let-7a and let-7d, for its numerous citations in the literature. Two additional microRNAs were also selected: let-7e, for its prognostic value at C0, C2 and C4 and miR-21, for its numerous citations in the literature. No microRNAs were included in the study and the potential of these microRNAs as biomarker are statistically evaluated.

E965
COMBINED CHEMOTHERAPY PLUS RADIATION THERAPY IS MORE EFFECTIVE IN LIMITED-STAGE DIFFUSE LARGE B-CELL LYMPHOMA OF THE TONSIL.
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Background: Primary extranodal non-Hodgkin’s lymphomas of the head and neck account for 10-20% of all non-Hodgkin’s lymphomas. Primary tonsillar lymphoma accounts for less than 1% of head and neck malignancies, although the tonsil is the most common primary extranodal site of head and neck non-Hodgkin’s lymphomas.

Aims: The purpose was to evaluate the prognostic factors and treatment outcome of patients with diffuse large B-cell lymphoma (DLBCL) of the tonsil.

Methods: In all, 114 patients with DLBCL of the tonsil with stage I or stage II, treated at multicenter in Korea, from September 1995 to April 2011, were included. The median age was 59 years and the majority of patients (61%) were male. Systemic symptoms were present in 6% of patients. International prognostic index (IPI) score was 0 in 54 patients (48%), 1 in 40 (35%), 2 in 14 (12%), and 3 (3%). Ten patients (8%) showed elevated level of lactate dehydrogenase (LDH). Treatment consisted of a combination of chemotherapy (CTx) and radiotherapy (RTx) for 38 patients (34%) and 72 patients (65%) received CTx only. Among those receiving RTx, the median RTx dose was 39 Gy. Results: After median follow-up of 32 months (range:0.4-106 months), event free survival (EFS) and overall survival (OS) were 25.9% and 42.5%, respectively. Significant prognostic factors included: age (≥ 60 year-old vs <60 year-old), LDH level (> upper normal limit and supper normal limit), IPI score (0-1 vs 2-3), and treatment (CTx plus RTx vs CTx only). On multivariate analysis: LDH level (hazard ratio [HR], 10.52; 95% confidence interval [CI], 2.548-43.449, p=0.001) and treatment (HR, 12.393; 95% CI 2.151-71.410) were independent prognostic factor of EFS and age (HR, 8.92; 95% CI 1.089-73.053, p=0.043), LDH (HR, 8.316; 95% CI 1.914-36.127, p=0.005), and treatment (HR, 8.943; 95% CI 1.089-73.425) retained statistical significance in OS.

Summary/Conclusions: MF tumoral cells express PD-1 protein in a high proportion of cases being a potential therapeutic target. Advanced disease, age ≥60 years and the degree of atypia of the tumoral infiltrate had an impact on survival.

E966
Abstract withdrawn.

E967
SEQUENTIAL TREATMENT WITH BENDAMUSTINE, RITUXIMAB AND DEXAMETHASONE FOLLOWED BY RITUXIMAB CONSOLIDATION AND LENALIDOMIDE MAINTENANCE FOR FRAIL ELDERLY PATIENTS WITH AGGRESSIVE B-NON HODGKIN LYMPHOMA
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Background: Frail elderly patients with aggressive B non-Hodgkin Lymphoma (a-B-NHL) in most cases show comorbidities such as to preclude the use of antracycline-based standard regimen. Although significant advances have recently been achieved in the therapy of older patients with a-B-NHL, there is still need for treatment strategies able to overcome the impact of drug toxicity on elderly frail patients.

Aims: The safety and efficacy of bendamustine and rituximab plus dexamethasone (RD-Benda) regimen were prospectively investigated in 14 elderly and frail patients with newly diagnosed a-B-NHL.

Methods: A plasma biobank from DLBCL patients was set up at the Centre Hospitalier Universitaire (CHU) UCL Namur Yvoir, Belgium (ethical agreement number B039201419613). Informed consents of all patients were obtained. In this way, blood samples from patients were taken before any treatment (C0), at the administration of the second and the fourth chemotherapeutic cure (C2 and C4) and at the remission review (Cf). In the case of a autograft, a sample was taken at the post-graft review (Cpg). The first step of this study was the selection of the microRNAs that will be quantified in all the samples of the biobank and that would potentially be used as biomarker. To this end, a quantification of 377 microRNAs was performed by TaqMan® Low Density Array on the plasma samples of two selected DLBCL patients and one healthy donor with no history of cancer. These DLBCL patients were selected based on their highly different response to treatment. One of them obtained a complete remission after a CHARTx treatment, while the other presented a refractory disease to the same treatment. Thereafter, we determined some criteria to use in a scoring system to evaluate their potential as biomarkers. In this way, one point was given to a microRNA each time it meets the criteria enabling it to be defined as a potential diagnostic, prognostic and/or remission biomarker. In addition, we define a disease progression biomarker of an inherent resistance to treatment, and/or biomarker of an acquired resistance to treatment.

Results: On the 377 microRNAs quantified into the plasma of the 3 selected donors (2 DLBCL patients and 1 healthy donor), 81 microRNAs were detected. Three microRNAs obtained the highest score of 5 points: miR-197, miR-20a and miR-451. Four points were attributed to miR-122, miR-19a and miR-19b. Two additional microRNAs were also selected: let-7e, for its prognostic value at C0, C2 and C4 and miR-21, for its numerous citations in the literature. No microRNAs were included in the study and the potential of these microRNAs as biomarker are statistically evaluated.

Summary/Conclusions: LDH level and age significantly influence outcome. A combined modality treatment, consisting of CTx and RTx, results in a satisfactory outcome in patients with stage I or II DLBCL of the tonsil.

E968
Abstract withdrawn.
Background: Sarcopenia is known to be associated with poor clinical outcome in patients with diffuse large B-cell lymphoma (DLBCL). There is no consensus concerning the optimal method to define sarcopenia in DLBCL.

Aims: In this study, given the uncertainty about the optimal SMI to define clinically meaningful sarcopenia in DLBCL, we compared the characteristics and clinical outcome between sarcopenic patients determined by L3 skeletal muscle index (L3-SMI) and those determined by pectoralis muscle SMI (PM-SMI) who were treated according to standard guidelines for 6-8 cycles. Patients in PR or with stable disease (SD) were grouped together for further analysis.

Methods: We retrospectively reviewed 193 DLBCL patients treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) therapy. Sarcopenia was classified by the region where the pretreatment skeletal muscle index (SMI) was measured.

Results: Both the sarcopenia-L3 and sarcopenia-pectoralis muscle (PM) groups had increased incidences of severe treatment-related toxicities and treatment discontinuation compared with the non-sarcopenia-L3 and non-sarcopenia-PM groups, respectively. The sarcopenia-L3 and non-sarcopenia-L3 groups had 2-year overall survival (OS) rates of 40.5% and 67.8% (P=0.001), respectively. The sarcopenia-PM and non-sarcopenia-PM groups had 5-year OS rates of 35.9% and 69.0% (P=0.001), respectively. When the sarcopenia-L3 alone and sarcopenia-PM alone groups were compared, there were no differences in baseline characteristics, treatment toxicity, or survival. In multivariate analysis, when compared with the non-sarcopenia-both group, OS was significantly worse in the sarcopenia-both group (HR, 2.480; 95% CI, 1.284-4.792; P=0.007), but not in patients with either sarcopenia-L3 alone or sarcopenia-PM alone (P=0.151).

Summary/Conclusions: L3- and PM-SMIs are equally useful to define sarcopenia, which is related to intolerance to R-CHOP therapy and to worse survival in patients with DLBCL. More prognostic information can be obtained when these two SMIs are combined to define sarcopenia.
E970
HIGH COMORBIDITY INDEX ALONG WITH HIGH NCCN-IPI STRONGLY INFLUENCE SURVIVAL OF DIFFUSE LARGE B CELL LYMPHOMA PATIENTS: SERBIAN LYMPHOMA GROUP EXPERIENCE

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Background: A few studies have validated the prognostic significance of the NCCN International Prognostic Index (NCCN-IPI) so far. However, some patients with low risk according to NCCN-IPI have poor survival, and thus clinical parameters, that might better characterized patients within risk groups, need to be explored.

Aims: The aim of this study was to evaluate prognostic significance of current indexes such as International Prognostic Index (IPI), NCCN-IPI, and the influence of comorbidities on the overall survival (OS) of patients with newly diagnosed diffuse large B cell lymphoma (DLBCL).

Methods: A total of 708 patients (383 males/345 females) with the median age of 58 years (range 18-89) were included in the study. Majority of patients received R-CHOP (Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, Prednisone) protocol, 652 (92.1%), while 29 (4.1%) received R-EPOCH (Rituximab, Etoposide, Cyclophosphamide, Doxorubicin, Vincristine, Prednisone), and 27 (3.8%) received R-CVP (Rituximab, Cyclophosphamide, Vincristine, Prednisone).

Results: According to the Ann Arbor classification, stage I and II had 332 patients (46.9%), while stage III and IV had 376 patients (53.1%). Bulky disease was present in 201 patients (28.4%), and B symptoms in 437 patients (61.7%). Poor European Cooperative Oncology Group (ECOG) performance status (≥2) had 145 patients (20.5%). Bone marrow involvement was present in 97 patients (13.7%). At least one comorbid condition had 309 patients (43.6%), while high Charlson Comorbidity Index (CCI) had 44 patients (6.2%). Majority of patients had cardiovascular disorders (223, 31.5%), endocrinological (63, 8.9%), neurological (20.2, 8.9%), previous malignancy (19, 2.7%), pulmonary (18, 2.5%), psychiatric (13, 1.8%), nephrotic (8, 1.1%), autoimmune (6, 0.8%), and other (13, 1.8%). According to IPI, low, low intermediate, high intermediate and high risk had 332 patients (46.9%), 174 (24.6%), 132 (18.6%), and 70 (9.7%), respectively, while according to NCCN-IPI, 133 (19.6%) patients had low risk, 335 (47.3%) low intermediate, 198 (28.0%) high intermediate, and 36 (5.1%) high risk. Overall treatment response (ORR) was achieved in 615 patients (86.9%). Disease relapse was confirmed in 116/615 patients (18.9%). The patients with B symptoms (Log Rank=1.50, p=0.0001), and bulky disease (Log Rank=1.79, p=0.0001) had inferior OS compared to those without B symptoms or bulky disease. All parameters incorporated in IPI, as well as in NCCN-IPI, were significantly associated with OS (p<0.01). Moreover, the patients with at least one comorbid condition had inferior OS (Log Rank=5.41, p=0.20), as well as those with high CCI ≥2 (Log Rank=7.59, p=0.006). Regarding OS, IPI (Log Rank=97.36, p<0.0001), and NCCN-IPI (Log Rank=102.29, p<0.0001) confirmed its prognostic significance. Furthermore, the patients with high CCI had significantly inferior median OS in the high risk group according to IPI (19 months vs 37 months), and NCCN-IPI (12 months vs 19 months).

Summary/Conclusions: NCCN-IPI represents useful prognostic index in DLBCL patients, and can better describe patients within risk groups, compared to IPI. Moreover, comorbidities contribute to inferior survival through frailty, drug dose reduction and poorer tolerability.
E972
POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS: A SINGLE-CENTER CASE SERIES
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Background: Post-transplantation lymphoproliferative disease (PTLD) is a complication of both solid organ transplant (SOT) and haematopoietic cell transplant (HCT) and represent a very heterogeneous group.

Aims: The objective of this study is to evaluate the epidemiology, clinical features, characterization and therapeutic management of this disease.

Methods: We evaluated a total of 52 patients diagnosed between May 1995 and February 2017. We analyzed the following data: type of transplantation, immunosuppression time from transplantation to lymphoma diagnosis, clinical stage, response to treatment and survival. We classified them morphologically according to the WHO criteria (2016 revision), using immunohistochemical and molecular techniques and their relationship with the Epstein-Barr virus (EBV) by studying the expression of EBERs. We correlated clinical data with morphological, immunohistochemical and molecular results.

Results: Among the 52 patients, 31 were men (59.6%) and 21 women. PTLD after SOT were 45 (86.5%), of which 16 were after liver transplant (35.6%), 14 cardiac (31.1%), 9 pulmonary (20%), 4 renal (8.3%) and 2 double (cardiac-pulmonary and cardiac-renal) (4.4%). There were 7 PTLD after HCT, 2 identical HLA family donor, 2 unrelated donor, 2 dual umbilical cord blood and 1 autologous. Of the 52 PTLD, 48 were B lymphomas (92.3%), of which 26 were diffuse large B-cell lymphomas (DLBCL) (54.2%), 7 polymorphic (14.6%), 7 low-grade (14.6%), 4 Burkitt lymphomas (8.3%), 1 Hodgkin’s lymphoma (2.1%) and 1 non-classifiable. Other 4 PTLD were T lymphomas (8.7%), 2 anaplastic, 1 T/NK lymphoma, and 1 gamma/delta T lymphocytosis. 35/52 PTLD were EBV + (67.3%). The median time of immunosuppression was 123 months in renal transplant, 93 months in liver, 85.5 months in cardiac, 51 months in lung and 3 months in HCT. Histologically, it was 96 months in T lymphomas and 80 months in B lymphomas, being 51 months in EBV + and 124 months in EBV−. Fifty percent of Burkitt lymphomas were diagnosed after lung transplant, while 85% of low-grade lymphomas were diagnosed after liver transplant. Clinical stage was Ill/IV in 73% of the patients (38). Among the 52, 45 received treatment (86.5%), 37 with immunochemotherapy (82.2%) and 8 with Rituximab (17.8%). Three patients responded to reduction of immunosuppression (5.8%) and 3 did not receive any treatment for early death (5.8%). At the time of writing, 19 patients remain alive (36.5%) and 33 have died. The median survival of these patients was 19.5 months (0-198).

Summary/Conclusions: PTLD constitute a very heterogeneous group. Its appearance is much earlier in the HCT than in the SOT and, within this latter group, it is earlier after lung transplant and later after renal transplant. The most common type in our series is DLBCL. The majority are related to EBV, so post-transplant monitoring is essential, and its diagnosis is earlier than in EBV−. Most low-grade lymphomas appear post-liver transplant, either in relation to viral pathogens or autoimmune diseases. Survival is significantly lower than in other primary LPS. -AR-SA-We analyzed the following data: type of transplantation, immunosuppression used in both induction and maintenance, immunosuppression time from transplantation to lymphoma diagnosis, clinical stage, response to treatment and survival. We classified them morphologically according to the WHO criteria (2016 revision), using immunohistochemical and molecular techniques and their relationship with the Epstein-Barr virus (EBV) by studying the expression of EBERs. We correlated clinical data with morphological, immunohistochemical and molecular results.

E973
SURVIVAL OUTCOMES AFTER FIRST LINE THERAPY IN DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) USING A UNITED STATES (US) ELECTRONIC MEDICAL RECORD (EMR)-BASED COHORT
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Background: In the rituximab era, the recommended first-line therapy (1LT) in DLBCL patients who can tolerate combination therapy is rituximab combined with chemotherapy. For refractory/relapsed disease, high-dose chemotherapy with stem cell transplant, combination chemotherapy, or single-agent rituximab are considered. While the efficacy of rituximab has been shown in clinical trials, few studies have evaluated survival outcomes in patients seen in routine clinical care.

Aims: We evaluated survival outcomes in a US population of newly diagnosed DLBCL seen in routine clinical care.

Methods: In this retrospective study, adult patients ≥18 years old with newly diagnosed DLBCL were identified from the Humedica, a large US electronic medical record database, between 01/01/08 and 07/31/15. DLBCL diagnosis was determined by the presence of ≥1 inpatient record or ≥2 outpatient records with DLBCL diagnosis codes; the first DLBCL record served as the index date. Following the index date, initiation of 1LT for DLBCL was required. For the assessment of the survival outcomes, patients were evaluated from the date of treatment initiation until death, loss to follow-up, or end of study (09/30/15). Median progression-free survival (PFS) (defined as initiation of second-line therapy, evidence of supportive care >30 days after the end of a line of therapy, or death), median overall survival (OS), and PFS and OS rates at 2 years following initiation of 1LT were evaluated using unadjusted Kaplan-Meier analyses.

Results: 1,436 newly diagnosed DLBCL patients who initiated 1LT met the patient selection criteria. 54.0% were male, and the mean age was 66.4 years (SD: 13.7). At baseline, 27.4% of patients had a Charlson Comorbidity Index of ≥2, and the most common comorbidities were diabetes (20.3%), chronic pulmonary disease (15.5%), and moderate to severe renal disease (9.5%). In 1LT, 92.1% of patients received combination therapy, with R-CHOP (63.5%) being the most common combination therapy. 7.9% of patients received monotherapy upfront, with rituximab (77.2%) being the most commonly used single agent. At 2 years following initiation of 1LT, the Kaplan-Meier OS and PFS were 79.2% and 67.3%, respectively. Median OS was not reached, and median PFS was 53.9 months (95% confidence interval: 45.2, 61.5). OS and PFS were also compared among patients receiving monotherapy vs combination therapy in unadjusted analysis. At 2 years, OS was 80.2% for patients receiving combination therapy vs 67.4% (P=0.0093) for patients receiving monotherapy. Also at 2 years, PFS was 68.3% for patients receiving combination therapy vs 55.1% (P=0.0051) for patients receiving monotherapy.

Summary/Conclusions: In this population of patients with newly diagnosed DLBCL receiving 1LT, survival outcomes at 2 years were significantly improved for patients treated with combination therapy vs monotherapy. Future analysis will explore the differences in clinical characteristics of patients treated with monotherapy vs combination therapy in the 1LT setting.
AN EXPERIENCE WITH LONG ACTING FACTOR VII PROPHYLAXIS IN PAEDIATRIC AND YOUNG ADULT PATIENTS WITH HAEMOPHILIA A
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Background: Hemophilia is an X linked inherited bleeding disorder. Recurrent Joint bleeds and muscle bleeds are the common manifestations leading to long term comorbidities in hemophilia. High dose factor prophylaxis has been proven to be very effective in preventing joint problems in western world. We look for a cost effective and feasible way for Indian patients in terms of reduced dose and frequency of factor infusion. Data on prophylaxis with low dose long acting factor infusion on twice weekly dosing schedule is limited.

Aims: To study the efficacy and safety of long acting factor VII (Elocate) for tertiary prophylaxis in pediatric and young adult patients with moderate and severe haemophilia A.

Methods: Thirty eight patients with moderate and severe haemophilia A without inhibitors and age range from 1 to 25 years were included in this study. Patients were initially observed for 4 months during which they received therapeutic doses of long acting factor VII, ELOCATE (Factor VII with Fc Fusion Protein) on episodic basis after clinical bleed. In next 4 months they received prophylactic ELOCATE, given intravenously at doses of 20 unit/kg body weight on twice weekly schedule. Annual bleeding rates, school absenteeism, emergency visits, aspects of quality of life and joint scores were compared during observation and prophylaxis period.

Results: Total number of bleeds during observation and prophylaxis period was 607 and 90 respectively. Annual bleeding rate was 47.9 during observation period and 7.1 during prophylaxis. There was 85.1% reduction in bleeding rates on prophylaxis. School/college absenteeism was 3.1 days/ month and 0.8 days/month during observation and prophylaxis respectively. Emergency visits were significantly more during observation. None of the patients developed inhibitors and two patients had superficial thrombophlebitis during prophylaxis.

Quality of life assessment using KIDSCREEN QOL questionnaire showed moderate to marked improvement in quality of life domains during prophylaxis.

Summary/Conclusions: Low dose, twice a week, long acting factor VIII prophylaxis can be a reasonable option for patients with haemophilia A in developing countries. It significantly reduces joint bleeds, school absenteeism, Joint scores significantly without risk of inhibitor formation and also improves all domains of quality of life.

NOVEL MUTATIONS IN THAI CHILDREN WITH CONGENITAL FACTOR VII DEFICIENCY
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Background: Congenital factor VII (F7V) deficiency is a rare autosomal recessive coagulation disorder resulted from mutations in the F7V gene (F7). The disease severity is not correlated with F7V levels but might be determined by molecular mechanisms in F7.

Aims: To delineate the phenotypic and genotypic characteristics of patients with congenital F7V deficiency.

Methods: We described demographic data, clinical manifestations, and outcome of patients with congenital F7V deficiency. F7V mutation analysis was performed by PCR-direct sequencing.

Results: Of the ten patients diagnosed with F7V deficiency, five (50%) were males. The median age (range) at diagnosis was 19 days old (1-730). Consanguinity was found in 50% of the patients. Of the nine patients (90%) classified as severe, six patients presented with intracranial hemorrhage within the first month of life, two presented with gastrointestinal bleeding and one presented with hemorrhathrosis. There were eight different alterations identified. Four have been previously reported (c.1091G>A (p.R364Q), c.1238G>A (p.R413Q), c.1256C>T (p.T419M), and c.681G>T (IVS6+1T)). Four novel (c.1192G>T (p.D398Y), c.1313G>T (p.G420V), c.291+2T>C (IVS3+2T>C), and IVS6-2A>G) were identified with hemarthrosis. There were eight different alterations identified. Four have been previously reported (c.1091G>A (p.R364Q), c.1238G>A (p.R413Q), c.1256C>T (p.T419M), and c.681G>T (IVS6+1T)). Four novel (c.1192G>T (p.D398Y)), c.1313G>T (p.G420V), c.291+2T>C (IVS3+2T>C), and IVS6-2A>G) were associated with major bleeding especially during infancy.

Summary/Conclusions: This study reported Thai children with congenital F7V deficiency presented with life-threatening bleeding especially in the first year of life. Pathogenic including newly identified variants in the F7V gene were detected in all cases. Genetic counseling can be appropriately provided to reduce the risk of disease recurrence in the families at risk.

RETROSPETIVE EVALUATION OF PHENOTYPE AND MANAGEMENT OF A-HYPO-FIBRINOGENEMIA IN A COHORT OF ITALIAN PATIENTS
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Background: A fibrinogenemia (AF) and hypo-fibrinogenemia (HF) patients (pts) experience hemorrhages or thromboses, and the clinical management can be difficult.

Aims: To obtain information on AF/HF clinical phenotype and management.

Methods: This is a spontaneous retrospective, multicenter national study. Data are collected from clinical records.

Results: 2 AF and 12 HF pts have been enrolled (6M, 8F). Median follow-up: 39 months (1-553). Median fibrinogen activity/antigen level: 78mg/dL (0-150)/73mg/dL (0-140). Five pts experienced epistaxis, hematomas, ecchymoses, menometrorrhagia, intra-abdominal bleeding, gum hemorrhage, thrombosis. Fresh frozen plasma, fibrinogen concentrate (FC), cryoprecipitate, whole blood, tranexamic acid were administered in the majority of these events. One ischemic stroke, 1 lower limb arterial and 1 cerebral sinus thrombosis, 1 comitant anortic and inferior vena cava thrombosis occurred: 3 events during FC therapy 1 during puerperium. Heparin, low molecular weight heparin (LMWH), anti-platelet agents, fibrinolytic agents, warfarin were then administered. One gastrectomy, 1 lower limb amputation, 5 gynecological, 1 otorhinolaryngological and 1 plastic surgery were performed in 2AF and 3 HF pts: in AF leg amputation, in HF leg amputation, in HF 1000 ml bleeding was observed after 2 surgeries performed without prophylaxis. Eight pregnancies were initiated in 3 HF women. Two spontaneous deliveries (SD) and 2 cesarian sections (CS) were performed; 4 abortions occurred. FC prophylaxis and LMWH were administered during pregnancy in 3 and 4 cases, respectively. One venous thrombosis, 2 hemorraghes, 1 DIC and 4 complicated pregnancies were recorded. FC was administered at delivery and LMWH during puerperium, for the 2 CS. No complications at delivery occurred.

Summary/Conclusions: AF and severe HF pts experience severe hemorrhagic/thrombotic events. The intervening clinical situations are difficult to manage. Further large scale data collections are necessary in order to provide useful information to better characterize and manage patients suffering from these rare diseases.

RETROSPETIVE REVIEW OF FOUR DAYS OF VON WILLEBRAND’S FACTORS AS SURGICAL PROPHYLAXIS IN VON WILLEBRAND’S DISEASE
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Background: Von Willebrand disease (vWD) is the most common inherited bleeding disorder that manifests as easy bruising, mucocutaneous bleeding and excessive hemorrhage with invasive procedures. In 2007, Humate-P, a lyophilized concentrate of purified VWF and FVIII, was approved in the United States for treatment and prophylaxis. Current guidelines per National Heart, Lung, and Blood Institute (NHLBI) Expert Panel recommend 40-60 U/kg pre-operatively, followed by maintenance dose of 20-40 U/kg for 7-14 days for major surgery and 5 days for minor surgery. Here, we report a single-institution experience of a short course of Humate-P as surgical prophylaxis.

Aims: To assess if an abbreviated schedule of Humate-P given as perioperative dose of 40 U/kg for 2 days (one dose pre-op and one post-op) for peri-operative dental procedures and for 4 days (one dose pre-op and for 3 days post-op) for minor and major surgical procedures as surgical prophylaxis would result in equi-efficacious hemostasis without compromising patient outcomes.

Methods: We retrospectively identified 202 patients with known diagnosis of vWD at our institution that underwent surgical procedures requiring prophylaxis between 2002-2017. Ninety elective surgical events occurred among these patients that required peri-operative prophylaxis with Humate-P. These patients were treated with peri-operative dose of 40 U/kg on D0-1 for extensive dental procedures and for 4 days (one dose pre-op and one post-op) for minor and major surgical procedures as surgical prophylaxis would result in equi-efficacious hemostasis without compromising patient outcomes.

Results: 8 AF and severe HF pts experience severe hemorrhagic/thrombotic events. The intervening clinical situations are difficult to manage. Further large scale data collections are necessary in order to provide useful information to better characterize and manage patients suffering from these rare diseases.

Disclosure: All authors have declared no conflicts of interest.
NOVEL AND RECURRENT F7 MUTATIONS IN KOREAN PATIENTS WITH F7 DEFICIENCY WERE IDENTIFIED BY DIRECT SEQUENCING ANALYSES OF ALL EXONS AND FLANKING INTRONIC SEQUENCES. VARIANTS WERE ASSIGN according to the recently released criteria of 2015 ACMG standards and guidelines. Flanking intronic sequences. Variants were assigned according to the recently released criteria of 2015 ACMG standards and guidelines.

Methods:

Aims: To characterize F7 gene mutational patterns of Korean patients with coagulation Factor VII deficiency including their clinical and laboratory variability.

Methods: F7 gene mutations of total 16 unrelated Korean patients with Factor VII deficiency were identified by direct sequencing analyses of all exons and flanking intronic sequences. Variants were assigned according to the recently released criteria of 2015 ACMG standards and guidelines.

Results: A total of 14 mutations (pathogenic or likely pathogenic) were detected including four novel mutations (Glu66Lys, c.681+3A>T, Glu66Alafs, Ile290del).

Summary/Conclusions: Correlation of genetic data with coagulation laboratory and clinical findings suggested the presence of modifiers, which warrants further investigation in a larger cohort of patient for better clinical prediction and management in this rare bleeding disorder.

E978

AUDIT ON MANAGEMENT OF HIGH INTERNATIONAL NORMALIZED RATIO (INR) IN WARFARINISED INPATIENTS

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Background: Warfarin is the commonest used oral anticoagulant with an effective anticoagulant. The British Committee for Standards in Haematology guidelines recommend administration of 25-50µg of four factor Prothrombin Complex Concentrate (PCC) and intravenous (IV) Vitamin K 5mg for patients with major bleeding. 1-3mg of Vitamin K intravenously for those with minor bleeding and 1-5mg of Vitamin K orally for patients with INR >8 and who have no signs of bleeding. The aim of this audit was to compare our hospital’s performance against the above guidelines.

Methods: A total of 76 patients admitted between 01/08/2015-31/01/2016 were analysed retrospectively. Results: There were 103 incidents with INR level 5-8 and 24 with INR >8 in these 76 inpatients. Bleeding was documented in 18/127 cases, which included 6 incidents of major and 12 incidents of minor bleeding. In major bleeding, warfarin was withheld and Vitamin K administered. However, 4/6 (66.7%) of these patients got a dose different to 5mg advocated. Also, PCC was prescribed in only 50% of these patients. While 9/12 (75%) patients with minor bleeding received Vitamin K, only 3 of these 9 patients received the recommended dose of 1-3mg IV. Vitamin K was unnecessarily given to 9/12 (75%) patients with minor bleeding and 1-5mg of Vitamin K orally for patients with INR >8 and who have no signs of bleeding.

Summary/Conclusions: Our audit highlighted that there is less than 100% compliance in the recommended dose and route of vitamin K administration. A flowchart containing the guidelines will be designed to improve the management of high INR. To increase the awareness of this issue, teaching sessions for junior doctors and nursing staff are planned. A re-audit will be conducted once these steps are in place.

E979

NOVEL AND RECURRENT F7 MUTATIONS IN KOREAN PATIENTS WITH COAGULATION FACTOR VII DEFICIENCY

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Background: Coagulation factor VII deficiency is one of rare hereditary bleeding disorders with relatively limited clinical and genetic data. This study aimed to characterize F7 gene mutational patterns of Korean patients with coagulation Factor VII deficiency including their clinical and laboratory variability.

Methods: F7 gene mutations of total 16 unrelated Korean patients with Factor VII deficiency were identified by direct sequencing analyses of all exons and flanking intronic sequences. Variants were assigned according to the recently released criteria of 2015 ACMG standards and guidelines.

Results: A total of 14 mutations (pathogenic or likely pathogenic) were detected including four novel mutations (Glu66Lys, c.681+3A>T, Glu66Alafs, Ile290del).

Summary/Conclusions: Correlation of genetic data with coagulation laboratory and clinical findings suggested the presence of modifiers, which warrants further investigation in a larger cohort of patient for better clinical prediction and management in this rare bleeding disorder.
**Utility of CD157 in a FLAER based single tube five color combination for screening of paroxysmal nocturnal hemoglobinuria clone.**

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**Background:** Fluorescent Aerolysin (FLAER) based flow cytometric analysis of polymorphs and monocytes is the gold standard for the screening of paroxysmal nocturnal hemoglobinuria (PNH) clone. In recent years CD157 has been identified as a PNH marker which targets both polymorphs and monocytes. It can be used in a single tube five color combination to screen polymorphs and monocytes simultaneously.

**Aims:** The objective of this study was to analyse the utility and advantage of CD157 in the PNH screening along with its ability to replace CD24 and CD14.

**Methods:** Our routine protocol for PNH screening included single tube six color antibody cocktail in following combination: FLAER-AF488, CD24-PE, CD15-PerCPCy5.5, CD14-PeCy7, CD64-APC, CD45-APC H7. We assessed the utility of single tube 5 color combination of FLAER-AF488, CD15-PE, CD15-PerCPCy5.5, CD64-APC, CD45-APC H7 for PNH screening and compared the results with the routinely used 6 color panel. Laboratory cutoff for CD157 was defined by running 10 samples from healthy individuals. Sensitivity analysis was assessed in spiking experiments by diluting a PNH positive sample with large clone size in a serial 10 fold dilution. Inter assay and intra assay precision analysis was done by running samples in triplicates across different clone size range and calculating the coefficient of variance (CV). Correlation of PNH clone size obtained from CD24/CD14 and CD157 was assessed by analysing a total of 30 samples across a wide range of PNH clone size (0.06-97.3%).

**Results:** CD157 was sensitive at the level of 10-4 and better. Frequency of cells with PNH phenotype in normal samples were found to be <0.002%. The CVs of intra-/interassay precision analysis ranged from 0.92/6% to 3.2/4/4% for granulocytes and 1.92/5 to 5.3/6/5 for monocytes. The PNH clone size, as obtained by CD157 based analysis was highly comparable to those obtained by CD24/CD14 based assay (R2>0.993). CD157 was found much better than CD24/CD14 in identifying the type II PNH clones. There was no false positive or false negative result. The cost of analysis was found to be approximately 15% lesser than the routinely used 6 color assay.

**Summary/Conclusions:** CD157 is a robust, reliable and potentially useful universal marker for PNH screening. Its inclusion in a single tube five color FLAER based panel is a cost effective approach which is ready to replace CD24/CD14 from routine PNH screening.

Aims: Here we report data on the clinical management and treatment results of patients with PNH undergoing surgery.

Methods: We collected data on 14 surgical interventions of 11 patients (8 males; age, 25-76 years). All patients had a high prevalence of PNH clone cells (55-99% in PMN) and were receiving eculizumab (ECU). Types of surgery were: 6 laparoscopic cholecystectomies, a transjugular intrahepatic portosystemic shunt, a distal splenorenal shunt, a laparoscopic Achilles allograft ligation, a gastrectomy, an emergency appendectomy, and 3 urologic interventions. Ten patients received ECU 900mg, while one (patient E, surgery 6) received 1200mg since he had developed hemolysis at a previous surgical intervention (surgery 5). In two cases (patient G, surgery 8; patient H surgery 11), an additional dose of ECU was administered before surgery. Patient H (surgery 11) had developed hemolysis at previous surgical interventions (surgeries 9 and 10). In most cases, either the date of the ECU dose was taken into account when scheduling surgery or the ECU dose was moved forward to coincide with the date of surgery. The time between the last ECU dose and surgery was normally one day (range, 1-8).

Results: In nine cases, transfusions were required due to hemorraghic complications. Patient I (surgery 12) had a thrombotic event leading to acute myocardial infarction one week after surgery. Increased hemolysis was observed (increased LDH and/or presence of hemoglobinuria) in five cases (patients E, H, I and K; surgeries 5, 9, 10, 12 and 14) during the week following surgery. Two of these patients (patients E and H) later underwent additional surgery (surgery 6 and surgeries 10 and 11, respectively). The pre-surgical ECU dose was increased in surgery 6 (patient E) and an extra dose was administered in surgery 11 (patient H) and no hemolysis was observed. (See Table 1).

Summary/Conclusions: Our findings lead us to recommend to perform the intervention within 24 hours of the administration of Ecu in programmed surgery for which it is necessary to program the dose. While in urgent surgical interventions put a new dose on the day of the intervention independently of the previous dose, also the normal ECU dose could be increased or an extra dose be administered in order to minimize the risk of hemolysis in high-risk patients or those with a previous history of surgery-related hemolysis.

E984

EFFICACY OF ECUILIZUMAB IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) PATIENTS WITH OR WITHOUT APLASTIC ANEMIA: PROSPECTIVE, LONGITUDINAL STUDY OF KOREAN PNH COHORT


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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a hematopoietic stem cell disease characterized by the intravascular lysis of red blood cells. PNH patients often have underlying bone marrow failure (BMF), with aplastic anemia (AA) as the most frequently associated type. Eculizumab, a humanized monoclonal antibody that binds specifically to human complement protein C5, has been used in Korea since 2012.

Aims: The purpose of this study was to determine whether eculizumab-treated patients show clinical benefit and reduced risk of complications regardless of concomitant AA in a Korean population.

Methods: Forty-six PNH patients ≥18 years of age diagnosed by flow cytometry and treated with eculizumab for more than 6 months were analyzed in the prospective Korean PNH registry. Patients were categorized into two groups: PNH patients with concurrent AA (PNH/AA) and without (classic PNH). Patients with severe AA/PNH were excluded. Biochemical indicators of intravascular hemolysis, hematological laboratory values, transfusion requirement, and PNH-associated complications assessed by the treating physician were reported every 6 months after enrollment.

Results: The median age of the study population was 49 years (range, 18-73 years) at eculizumab initiation and the median duration of eculizumab treatment was 34 months (range, 6-44 months). Median LDH fold x upper limit of normal was 7.29 (range 2.4-23.7) and GPI-deficient granulocytes was 92.8% (range, 15.7-100%) at the time of eculizumab treatment. PNH-related signs and symptoms were thromboembolism (TE, n=19), renal failure (n=20), pulmonary hypertension (n=5), and severe/recurrent abdominal pain requiring opioids (n=17). Of 46 total patients, 12 (26%) were classified as having PNH/AA and 34 with classic PNH. There were no substantial differences in laboratory findings, transfusion requirement, or clinical signs and symptoms of PNH between the two groups. Treatment with eculizumab induced a rapid inhibition of hemolysis. At the time of 6 months follow-up, LDH level decreased to near normal levels in all patients and this effect was maintained until 36 months follow-up regardless of concomitant AA. Mean hemoglobin level significantly increased from the first 6 months of eculizumab treatment and the effect (hemoglobin above 10 g/dL) was sustained throughout 36 months in both groups. Transfusion-independence was achieved by 54.3% within the first 6 months of treatment and 86.4% by the last 36 months (83.3% in PNH/AA vs 87.5% in classic PNH). The mean number of RBC units transfused was significantly reduced from 8.5 units during the previous 6 months to 1.6 units for the first 6 months in total PNH patients (Fig). There were no significant differences in clinical outcomes (ie, LDH and transfusion unit per every 6 months) with eculizumab between the two groups. All TE (n=19) patients in whom 6 received concomitant anticoagulation therapy were resolved on the eculizumab; one classic PNH patient had recurrence of TE at the same site after discontinuation of anticoagulation therapy while on eculizumab. Among 9 patients who had baseline eGFR less than 60 ml/min/1.73m2, 5 patients (56%) showed improvement of eGFR during the eculizumab treatment and 4 patients stabilized eGFR.
Summary/Conclusions: Clinical outcomes with eculizumab were significantly improved compared with the baseline in patients with both PNH/AA and classic PNH. This study demonstrated that eculizumab has a beneficial role in the management of patients with PNH/AA, similar to that of classic PNH, by inhibiting hemolysis and reducing transfusion requirements, thus resulting in the improvement of clinical signs and symptoms.

E985
DIAGNOSIS AND FOLLOW-UP OF THE CLONES OF PAROXYMAL NOCTURNAL HEMOGLOBINURIA BY FLOW CYTOMETRY
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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a very rare chronic disease associated with a clonal expansion of one or several hematopoietic stem cells carrying acquired somatic mutations of PIG-A gene resulting in GPIAP deficient blood cells and great susceptibility to complement mediated cell lysis. Diagnosis of PNH is of importance and flow cytometry (FC) is a required tool for this. We report 33 cases of PNH diagnosed and monitored by FC.

Aims: To show the interest of flow cytometry for the diagnosis and follow-up of PNH clones in some risky haemopathies.

Methods: A PNH clone has been researched in 234 patients since August 2006 to January 2017. The PNH clone was investigated for bone marrow aspira-

Results: Out of 234 cases analyzed, 201 cases (85%) showed absence of PNH clone and 33 cases (14%) had a PNH clone. There are 14 women and 19 men; Sex ratio (M/F) = 1.35, mean age = 42.27 years (17-73). Among patients that should be screened for positive PNH clone we have bone marrow failure: 25 positive (21.9%) of 114 cases screened, hemolytic anemia with negative direct coombs test (DCT), myelodysplasia (MDS), unexplained cytopenia and thrombosis. The search for the PNH clone by FC is based on the analysis of the following monoclonal antibodies: Fcar and CD59 with gating on CD45 for neutrophils; CD59 with gating on monocytes and CD559 with gating on Glycoporphin A for red blood cells. We judged that the patient has a PNH clone when the deficiency is > 0.5% on at least two markers highlighted on two different lines. FC surveillance is provided in the absence of a deficit or in cases of recurrence or single-line deficit.

Summary/Conclusions: Thus, in patients with AA the decrease of NK-T cell level was observed along with recovery of hemopoiesis in all the subgroup variants. Previously we have shown that the decrease of NK-T cells accompanies the increase of the clone in 28% of the cases and in 9% of the cases a thrombosis developed. This interest was confirmed by the study of B. Hochsmann who demonstrated in 155 cases of followed PNH, a significant increase of the clone in 28% of the cases and in 9% of the cases a new clone appeared. (2). The application of flow cytometry enabled us to make the diagnosis and to determine the phenotypic profile of PNH clones and to specify their sizes as well as to follow up the patients.

E986
ASSOCIATION OF T-, B-, NK AND NKT CELLS WITH THE DURATION, COMPLETENESS AND OTHER CHARACTERISTICS OF REMISSION IN PATIENTS WITH APLASTIC ANEMIA
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Background: Immune-mediated dysregulation of hemopoiesis is the basis for pathogenesis of aplastic anemia (AA). Dysbalance of T cell subsets, especially Th1 and Th2 and produced by them cytokines serves as a possible mechanism of this phenomenon. It is suggested, that NK-T cells play an important role in regulation of Th1:Th2 balance. The role of NK-T cells in development of aplasia of hemoipoiesis in AA now is broadly studied. Nevertheless, up to this moment, the features of balance of T lymphocyte subsets, and, especially NK-T cells during stable and prolonged remission are not characterized yet.

Aims: To evaluate the association of T-, B-, NK and NKT cells in AA patients with the duration of remission, its completeness, duration of period free of immunosuppressive therapy (IST) and the size of PNH-clone.

Methods: The studied group included 36 patients with AA in remission, reference group – 20 patients with primary diagnosed AA. Level of T-, B-, NK and NKT cells in peripheral blood (PB) and bone marrow (BM) was evaluated using 5-color flow cytometry (Beckman Coulter, FC-500).

Results: Group of AA patients in remission was divided into subgroups in four variants: 1) according to the remission duration (<12 months, 12-24 months, 24-36 months, >36 months); 2) by the completeness of remission (CR, PR); 3) duration of IST-free period (<1 year, ≥1 year); 4) PNH-clone size (0.1-1%, 1-10%, >10%). Levels of T-, B- and NK cells in AA patients with remission varied broadly in different subgroups, but there were not revealed any clear tendency in their dynamics in all assigned subgroups, except for NK cells. Significant AA patients the level of NK-T cells in PB and BM exceeded normal level 1.8- and 2.2-fold, respectively. In remission lasting less than 12 months NK-T cell level decreased significantly; then, along with increase of remission duration (24-36 months), it normalised, and in patients with remission ≥36 months it significantly decreased both in PB and BM (data presented in table 1). In patients with PR, as compared with primary AA patients, NK-T cells decreased 2.8- and 1.9-fold, respectively, and further in CR this tendency persisted. Duration of IST-free period less than 1 year and ≥1 year was also accompanied by a significant and stable decrease of NK-T cells in PB and BM. It is known that PNH-clone presence is a favourable factor for treatment response. Therefore, we are interested to study the appearance of NK-T cell level in AA remission with the size of PNH-clone. In subgroup with small PNH clone (0.1-1%) NK-T cell level was decreased as compared to primary AA patients, and it significantly decreased further along with growth of PNH-clone size.

Table 1. NK-T cell level (%) in patients with AA in remission according to subgroups.

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>NK-T cell level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR</td>
<td>1.2 (0.8-1.6)</td>
</tr>
<tr>
<td>CR</td>
<td>2.8 (1.8-3.2)</td>
</tr>
<tr>
<td>&gt;1 year</td>
<td>3.2 (2.0-4.4)</td>
</tr>
<tr>
<td>&lt;1 year</td>
<td>1.9 (0.8-2.8)</td>
</tr>
</tbody>
</table>

Summary/Conclusions: Thus, in patients with AA the decrease of NK-T cell level was observed along with recovery of hemopoiesis in all the subgroup variants. Previously we have shown that the decrease of NK-T cells accompanies the increase of the clone in 28% of the cases and in 9% of the cases a thrombosis developed. This interest was confirmed by the study of B. Hochsmann who demonstrated in 155 cases of followed PNH, a significant increase of the clone in 28% of the cases and in 9% of the cases a new clone appeared.

E987
A NOVEL DUAL-REAGENT SINGLE TUBE FLOW CYTOMETRIC ASSAY TO SCREEN PAROXYMAL NOCTURNAL HEMOGLOBINURIA
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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired hematopoietic stem cell disorder resulting from loss of membrane-bound glycosylphosphatidylinositol (GPI) anchor protein. The disease is characterized by heterogeneous clinical phenotypes including intravascular hemolysis, cytopenia(s), bone marrow hypoplasia and atypical site thrombosis. Screening guidelines recommend documentation of the lack of at least two GPI-related anti-gens on at least two cell lineages. Alexa fluo 488 conjugated fluorescent Aerolysin (FLAER-AF488) has become a mandatory component in FCM based PNH assays.

Aims: We have analyzed the feasibility of a novel dual-reagent assay for a simplified, single tube, cost-effective approach for PNH screening.

Methods: EDTA anti-coagulated peripheral blood of patients referred to department of Hematology with clinical suspicion of classical-PNH/ aplastic anaemia, was tested with a single tube panel of FLAER-AF488/CD33/4PC. Simultaneously, the routine two tube flow cytometry assay (established sensitivity of 0.1%) for PNH was performed on patients (28 of granulocytes and FLAER/CD33/CD114 for monocytes) was performed in the same sample. Each tube was run till a minimum of 50,000 granulocytes were acquired or till the tube ran dry. A cluster of at least 20 FLAER negative events was considered for reporting PNH-clone positivity in both granulocytes and monocytes. The granulocytes and monocytes anti-gens positivity and the respective clone sizes detected by both the strategies were compared.

Results: A total of 33 patients and 7 healthy controls were analyzed by both dual-reagent and conventional strategies. Among the thirty-three patients, twelve patients concurrently showed the presence of PNH clones by both methods. The only false positive was noted for granulocytes and monocytes by both strategies, indicating complete concordance at a sensitivity of 0.2% (Chi Square p<0.0001). Of the PNH positive cases, the mean PNH clone sizes among the granulocytes by dual-reagent and conven-
TREATMENT OF REFRACTORY APLASTIC ANEMIA WITH ELTROMBOPAG: EXPERIENCE OF A CENTER

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Background: Eltrombopag, a thrombopoietin receptor agonist, was approved in 2008 for the treatment of immune thrombocytopenic purpura. More recently, benefits demonstrated in the proliferation and maintenance of hematopoietic stem and progenitor cells (HSTC) led to its use and approval in the treatment of severe aplastic anemia (AA) refraactory to immunosuppressive therapy.

Aims: In this report, we evaluated response to eltrombopag in patients with refractory AA (Pearson’s r=0.991, p=0.000) and the monocyte PNH clone sizes (Pearson’s r=0.993, p=0.000) detected by both the analysis strategies.

Summary/Conclusions: This pilot study demonstrates the practical feasibility of a simple, cost-effective and widely applicable dual-reagent, single tube PNH-screening assay at a sensitivity of 0.2%. The study needs to recruit patients of various hematological disorders besides healthy controls, and although seems effective for analyzing classic and subclinical PNH, the strategy has to be further standardized to achieve a sensitivity of 0.01%.

Chronic lymphocytic leukemia and related disorders - Biology

DECREASED EXPRESSION OF ADHESION MOLECULES IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) CELLS OF PATIENTS TREATED WITH IBRUTINIB

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Background: The B-cell receptor (BCR) pathway in CLL plays a well recognized role in the onset and progression of the disease and the resulting development of mechanism-driven drugs is revolutionizing the therapeutic management. Ibrutinib is a BTK inhibitor that plays an important role in the BCR pathway and induces several alterations in CLL cells.

Aims: The modulation of the expression of adhesion molecules on the surface of CLL cells from patients treated with ibrutinib has been evaluated to analyze the effect of the treatment on the relationship between the microenvironment, that promotes cell survival and proliferation, and the leukemia cells with the consequent cell mobilization and increased drug exposure.

Methods: In a cohort of 101 CLL patients treated with ibrutinib (420mg/die) and rituximab (375mg/m2/week) in the GIMEMA LLC1114 trial, we evaluated, before and after 15 days of therapy, the surface expression on leukemic cells of several adhesion molecules. In detail, using 8 color antibody combinations (all from Becton Dickinson, BD, San José, CA) we evaluated the MIF expression (using the FACSQuant II, BD) of CD11a, CD18, CD34, CD40, CD43, CD44, CD49d, CD62L, CD62L, CD80, CD81, CD154, CD184, CD185 on CD5/CD19+ leukemic cells.

Results: The number of CD5/CD19+ did not increase after 15 days of treatment (52.8±58.8 vs 53.4±5.10×10^9/L; p=0.36) probably because of the concomitant rituximab administration, which ‘masks’ the mobilization effect induced by ibrutinib. We observed a significant down-modulation of CD62L (461±435 vs 171±148; p<0.0001), a molecule (L-selectin) that has been reported as the key factor controlling the binding of CLL cells to the endothelial walls in vivo. The CD69 expression resulted also significantly decreased (744±784 vs 438±716; p<0.0041), is expressed on CLL cells in the tissue microenvironment, both in the bone marrow and in lymph nodes. We observed the significant down-modulation of the expression of CD43 (3265±2282 vs 2515±1826; p=0.0063) and does therefore not seem a reliable marker in patients treated with ibrutinib. On the contrary, CD81 expression, another antigen utilized for MRD detection, resulted unchanged after 15 days of treatment. CD185 expression was significantly decreased (1502±1327 vs 804±587; p<0.001), while we unexpectedly observed the up-modulation of CD19 (224±2022 vs 318±1877; p<0.003; both antigens participate in the BTK signaling pathway). CD40, that interacts with activated CD4 T cells, resulted down-modulated (722±467 vs 395±262; p<0.0001). CD38 and CD49d, when expressed in >20% of the leukemic cells, resulted significantly decreased (74±395 vs 1±203; p<0.028 and p<0.021) down-modulated; both molecules have a role in the crosstalk between the leukemic cells and the microenvironment.

Summary/Conclusions: Within an ancillary biologic study of the GIMEMA LLC1114 protocol we observed a significant down-modulation in the expression of several adhesion molecules on the surface of CLL cells of patients treated with ibrutinib. Since these molecules promote the binding of the leukemic cells with the microenvironment, these results help to elucidate the mobilization process of CLL cells from the different compartments observed with ibrutinib and support its progressive efficacy over time in controlling the disease. A follow-up clinical analysis will define a possible correlation between these findings and response to treatment.
Background: The gene expression profile of chronic lymphocytic leukemia (CLL) cells revealed a homogeneous phenotype related to memory B cells accompanied by an aberrant expression of several proteins. For example, lipoprotein lipase (LPL), typically expressed in adipocytes, is readily detected in CLL cells. However, unlike their normal counterparts which are resting cells, CLL cells do proliferate. What energy source CLL cells use and which metabolic pathway they recruit is currently unknown. Because the gene expression profile of CLL cells is skewed towards that of adipocytes, and because they proliferate at similar rates, we hypothesized that like adipocytes CLL cells utilize free fatty acids (FFA).

Aim: Determine whether CLL cells are capable of utilizing FFA for energy production. (B) Determine whether lipid metabolism in CLL is LPL dependent. (C) Determine why LPL is aberrantly expressed in CLL cells.

Methods: Peripheral blood (PB) and bone-marrow derived lymphocytes were obtained from previously untreated patients with CLL. Imaging of CLL cells was done by electron microscopy, and PB lymphocytes were stained for Oil red O. Confocal microscopy studies helped in determining the cellular localization of LPL. To study the capacity of CLL cells to utilize FFA we developed an assay that measured the oxygen concentration in the sera of cultured CLL cells prior to and after adding FFA. In addition we measured the oxygen consumption of CLL cells derived from ibrutinib-treated patients. We used an immunoprecipitation (CHIIP) and luciferase assays to study the binding of STAT3 to the LPL promoter.

Results: To study whether CLL cells are capable of utilizing FFA we cultured 20 separate clones of cultured media-dissolved O$_2$ (dO$_2$) prior to and after adding FFA, assuming that if the cells oxidize the acid, dO$_2$ levels will drop. Indeed, after 48 hours incubation with FFA dO$_2$ levels were markedly reduced as compared with the dO$_2$ media levels of CLL cell incubated without FFA. Remarkably, unlike cultured PB lymphocytes and normal B cells, dO$_2$ levels of cultured CLL cells did not change. Intriguingly, the levels of dO$_2$ remained unchanged if CLL cells were incubated in the presence of FFA and ibrutinib. Similarly, the dO$_2$ levels of CLL cells obtained from ibrutinib-treated patients remained constant, suggesting that ibrutinib disrupts the capacity of CLL cells to utilize FFA. Oil Red O staining of CLL bone marrow smears detected lipid deposits and electron microscopy confirmed the presence of lipid vacuoles in the cytoplasm of peripheral blood CLL cells but not in normal B cells, suggesting that like adipocytes, CLL cells store lipids in intracytoplasmic lipid vacuoles. Similarly to adipocytes CLL cells express LPL which mediates the uptake of lipid particles into the cells and catalyzes the hydrolysis of triglycerides to FFAs. Indeed, we detected LPL in the cytoplasm ofnormal B and in the cytoplasm of CLL cells. Furthermore, using small interfering RNA (siRNA) we knocked-down LPL mRNA levels and found that LPL-siRNA reduced the capacity of CLL cells to utilize FFA, suggesting that the lipid metabolism in CLL is LPL dependent. Because STAT3 is constitutively active in CLL cells, and because the LPL gene harbors STAT3 binding sites, we sought to determine whether STAT3 activates the LPL gene. Indeed, transfection of luciferase reporter gene constructs driven by LPL promoter fragments into MM1 cells revealed that STAT3 activates the LPL promoter. In addition, ChIP confirms that STAT3 binds to these STAT3-shRNA downregulated LPL transcripts and protein levels, confirming that STAT3 activates the LPL gene.

Summary/Conclusions: Our data suggest that CLL cells undergo metabolic reprogramming and use strategies normally utilized by adipocytes. This process is driven by constitutively activated STAT3 and is inhibited by ibrutinib.

**E992**

INHIBITION OF ARGinine UPTAKE VIA hUMAN CATIONIC AMINO ACID TRANSPORTER-1 (CAT-1): A NOVEL APPROACH FOR CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) THERAPY

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Background: Interference with cancer metabolism by specifically restricting extracellular nutrients is a rapidly emerging field of research. A variety of tumor entities depend on the availability of the amino acid arginine since they have lost the ability to synthesize it endogenously. The systemic depletion of arginine, induced by the arginine-metabolizing enzymes arginase deiminase or arginine is currently explored clinically in phase I-II studies. An alternative, largely unexplored approach that seems to be more a potential available strategy would be to target the specialized cell membrane transporter proteins. Arginine uptake into cells is mediated by members of different solute carrier families (hCAT-1, hCAT-2A, hCAT-2B, hCAT-3; y+LAT1, y+LAT2, ATB0, and b0, +AT), which differ in expression and regulation between individual cell and tissue types.

Aim: We wanted to clarify (i) if CLL cells depend on exogenous arginine, (ii) which transporter is responsible for arginine transport in human CLL cells and (iii) if the reduction of arginine availability via knockdown of this transporter inhibits CLL cell growth and viability.

Methods: Experiments were performed with both, primary human CLL cells, isolated from highly leukemic peripheral blood, and immortalized CLL cell lines. Primary CLL cells were left unstimulated or were stimulated via Toll-like receptor 9. The expression levels of arginine transporters were determined by quantitative RT-PCR and Western Blot. Arginine uptake was measured by [3H]-arginine import, cell proliferation by [3H]-thymidine DNA incorporation and cell viability using propidium iodide staining in flow cytometry. The expression of hCAT-1 was downregulated in HG3 CLL cells using lentiviral shRNA technology. HG3_hCAT-1_knockdown cells were injected s.c. in NOD/SCID/gcnull mice and tumor growth was monitored.

Results: We show that primary CLL and immortalized CLL cell proliferation depends on the availability of extracellular arginine. Screening a large panel of individual CLL patient samples and different immortalized CLL cell lines demonstrated that hCAT-1, y+LAT1 and y+LAT2 are the predominantly expressed arginine transporters. Upon activation the expression level of hCAT-1 further increased significantly. As a consequence both, in primary and immortalized HG3 CLL cell lines, was inhibited by the CAT inhibitor N-ethylmaleimide. Lentinival downregulation of the hCAT-1 transporter in HG3 CLL cells resulted in a significant reduction of arginine uptake, associated with an inhibition of cell proliferation and viability in vitro. The corresponding in vivo data of tumor growth upon hCAT-1 knockdown in a murine xenograft model will be presented at the conference.

Summary/Conclusions: Our results demonstrate that the hCAT-1 transporter is a potential pharmacological target structure in CLL cells. Development of small molecule- or antibody-based inhibitors of hCAT-1 might lead to a novel therapeutic approach for the treatment of CLL.

**E993**

FMC R IS A NEGATIVE REGULATOR OF B-CELL RECEPTOR SIGNALING IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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Background: Chronic lymphocytic leukemia (CLL) cells frequently display features of anergic B cells, including reduced B-cell receptor (BCR) signaling capacity and downregulation of membrane IgM (mIgM). These features are particularly evident in freshly isolated peripheral blood (PB) CLL cells belonging to the indolent, M-CLL subset (Lanham S et al, Blood. 2003). The mechanism responsible for this phenomenon is still unclear, but chronic stimulation with autoantigens has been considered as a possible explanation because of the observation that BCR signaling capacity and mIgM expression can spontaneously recover in CLL cells following prolonged in vitro culture (Mockridge CI et al, Blood. 2007). An alternative explanation for this phenomenon is that these anergic features are induced by soluble IgM molecules, which are absent from standard cell culture media, and could interact in vivo with the leukemic BCRs through recently described intermembrane immunoglobulin interactions or by binding to the Fc receptor (FcR). The latter is highly overexpressed in CLL cells, particularly those belonging to the M-CLL subset (Li FJ et al, Blood, 2011). According to this model, FcR participates in regulating the CLL cell fate.

Methods: CLL cells were isolated from PB or lymph nodes (LN)s using standard procedures. FcR stimulation was done using pentameric human Fc fragment, whereas BCR stimulation was done using goat anti-human IgM or anti-human IgG F(ab)2 fragments. Cells were then knockdown by RNA interference using the Nucleofector system and solution VC-009 program. Surface FcR and IgM levels were measured by flow cytometry on gated CD19+/CD5+ cells.

Results: We recently reported that FcR stimulation results in activation of certain downstream BCR signaling pathways and increased CLL cell survival in vitro (Gobessi S et al, ASH 2016, abstract 2015). To investigate whether FcR regulates BCR signaling capacity, we analyzed activation of downstream signaling molecules in CLL cells that had been pretreated for one hour with FcR and then stimulated with an anti-Ig light chain antibody. Decreased phosphorylation of SYK, AKT and ERK occurred in the FcR-stimulated samples, suggesting that FcR negatively regulates BCR signaling in CLL cells. Consistent with this finding, we also observed that FcR knockdown by RNA interference resulted in greater activation of SYK, AKT and ERK in anti-IgM stimulated primary CLL cells. Because IL-4 was recently shown to decrease BCR signaling capacity and surface IgM expression on CLL cells (Aguilar-Hernandez MM et al, Blood. 2016; Guo B et al, Blood 2016), we next investigated whether it will have an opposite effect on FcR expression. Stimulation of CLL cells (n=7) for 48 hours with IL-4 resulted in a mean 2.4 fold reduction in surface FcR expression and a 3.9 fold increase in surface IgM expression compared to unstimulated cells (P<0.001 and P=0.016, respectively). Since IL-4 is produced by T cells, which typically interact with CLL cells in LNs, we next compared surface FcR and IgM expression in two paired LN and PB CLL samples. Interestingly, whereas FcR levels on the cells of patients treated with the combination of IL-4 and ibrutinib were downregulated, IgM expression was unchanged, whereas no difference was detected in the expression of surface IgM. To further understand the mechanisms through which IL-4 regulates BCR signaling, we compared BCR signaling capacity of CLL cells cultured for 48 hours in the pres-
ence or absence of IL-4. Most of the investigated samples in this series showed reduced surface FcγR expression and increased surface IgM expression after IL-4 treatment, but a few cases showed only reduced FcγR expression and no change in IgM expression. Interestingly, these samples also showed greater anti-IgM induced phosphorylation of SYK, PLCζ2, AKT and ERK, suggesting that downregulation of FcγR is the primary mechanism through which IL-4 regulates the BCR signaling capacity of PB CLL cells. FcγR is downregulated by IL-4 and shows reduced expression in LN CLL cells, which could represent a mechanism to allow CLL cells to respond more effectively to stimulation with antigen encountered in the appropriate context.

Summary/Conclusions: These data show that FcγR is a negative regulator of BCR signaling in CLL cells. Overexpression of FcγR could be at least in part responsible for the reduced BCR signaling capacity of PB CLL cells. FcγR is downregulated by IL-4 and shows reduced expression in LN CLL cells, which could represent a mechanism to allow CLL cells to respond more effectively to stimulation with antigen encountered in the appropriate context.

E994

TRANSCRIPTION FACTORS AND CHECKPOINT INHIBITORS EXPRESSION WITH AGE: MARKERS OF IMMUNOSENESCENCE?

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Background: Aging is characterized by a progressive decline in immune surveillance that favors tumor development in older patients. One mechanism used by malignant cells to escape immune surveillance is the upregulation of immune checkpoint inhibitors. Another process associated with aging is genetic or epigenetic modifications of tumor suppressor genes (TSGs).

Aim of the work: We have correlated an age-related 6q deletion and progression into a T cell lymphoproliferative disease, identifying the BACH2 gene as a candidate TSG. We thus examined the expression of specific transcription factors (BACH2 and PRDM1) and checkpoint inhibitors (PD-1 and PD-L1) in the T cells with the higher potential for their participation in immunosenescence.

Methods: Peripheral blood mononuclear cells were isolated from whole blood using Lymphoprep (Stemcell Technologies) density gradient centrifugation. Lymphocyte subsets (CD19+, CD3+CD4+; CD3+CD8+) were isolated for subsequent molecular analyses using the MACS® Technology (Miltenyi), with the purification of each lymphocyte subpopulation between 95%-99%. PD-1 (PDCD1), PD-L1 (CD274), IL-4, IFNG, BACH2 and PRDM1 mRNA transcripts were quantified using qRT-PCR. BACH2 and BLIMP1 (PRDM1) protein expression were examined by Western blotting.

Results: Blood samples were obtained from 60 healthy volunteers and 41 untreated B-cell chronic lymphocytic leukemia (B-CLL) patients (median 67yo). Healthy donors (HD) between the ages of 20 to 90 years subdivided into <50 yrs (median: 36yo) and >50 yrs (median: 65yo). BACH2 mRNA expression in the HD groups is significantly down-regulated in CD4+, CD8+ T cells and CD19+ B cells from the older HD group (p=0.0012; 0.0045 and 0.0367, respectively). BACH2 expression was further reduced in CD4+, CD8+ T cells and CD19+ B cells from CLL patients compared to HD well balanced for age (p=0.001; <0.0001 and 0.0043). PRDM1 mRNA expression was inversely correlated with BACH2 in CD4+, CD8+ T cells and CD19+ B cells (r=0.61; 0.71 and 0.85, respectively). Curiously, PRDM1 was – as expected - significantly up-regulated in CD4+ and CD8+ T cells ([p=0.0034; 0.0017] from B-CLL patients but not in their leukemic B cells. Western blotting analysis demonstrated that BACH2 and BLIMP1 (PRDM1) protein expressions in the T and B cell subpopulations were significantly correlated with transcript expression. BACH2 and BLIMP1 protein expressions were up-regulated in CD4+ PD1+ T cells. We also observed that BACH2 down-regulation is correlated with increased IL-4 mRNA expression (r=0.67) but not IFNG in CD4+ T cells. These observations suggest that BACH2 down-regulation in CD4+ T cells could enhance the expression of effector memory-related genes, particularly Th2, such as IL-4 and PRDM1. PD-1 mRNA expression was up-regulated in CD4+, CD8+ T cells (p=0.0153 and 0.0214) in the older HD group and also up-regulated in the T cells from B-CLL patients (p=0.0014 and 0.0023) when compared to age-matched HD population. High PD-1 mRNA expression was correlated with increased age in HD B cells (p=0.04) with a further increase detected in CD8+ B cells (p=0.001). We also observed an inverse correlation between BACH2 and PD-1 in CD4+, CD8+ T cells (r=0.62 and 0.68); and between BACH2 and PD-L1 in CD19+ B cells (r=0.66).

Summary/Conclusions: These data suggest that down-regulation of BACH2/PRDM1 and up-regulation of PD1/PD-L1 mRNA expression in major lymphocyte subsets from CLL patients and older healthy controls are significantly correlated with the aging immune cells and could be part of the immunosenescence process.

E995

T-CELL EXHAUSTED PHENOTYPE IS ENHANCED DURING DISEASE PROGRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: The different biological mechanisms leading the clinical progression of CLL from early stages are currently not fully elucidated. Different progression factors that show a higher probability of progression, such as IL-4 production, have been related to the pathogenesis of CLL. However, a recent study has still not able to identify an important proportion of patients that eventually progress. Clinical progression from early stages to an advanced CLL is associated with a certainly reduced acquisition of molecular changes that are not able to explain the fifty percent of the CLL cases progressing. CLL cells are dependent on survival and proliferative signals from the microenvironment and are able to evade immune anti-tumoral responses using different mechanisms, which is a crucial feature for cancer development. T-cell dysfunction is one of the main sources of impaired anti-tumor immunity. In CLL, T cells show functional defects and have increased expression of the exhaustion markers PD1, CD244 and CD137 compared to T cells from healthy individuals. Taking this into account, we hypothesize that changes in the microenvironment, and particularly in T-cell exhaustion component, are contributing to the clinical progression of CLL.

Aims: In order to explore the role of the immune system in the progression of CLL we studied the immunophenotype of T cells from CLL patients using paired samples at diagnosis and progression.

Methods: A total of 14 CLL patients (median age, 69 years; median time to progression of 29.5 months) and 6 patients diagnosed with CLL that did not experience clinical progression during a median follow up of 34 months were included in the study. Multicolor flow cytometry was performed in matched samples at two time-points: diagnosis and progression before treatment or diagnosis and follow-up. We studied T-cell differentiation status based on CD45RA and CCR7 expression and the inhibitory receptors PD1, CD244, CD160, LAG3, TIM3 and CTLA4. We also analyzed the expression of the transcription factors PD1/PD-L1, BACH2, and BLIMP1.

Results: We observed a significant increase in CD8* absolute numbers (P=0.0107) and a significant decrease of the CD4:CD8 ratio (P=0.0012) with progression. T cells increased their effector memory (EM) (CD45RA-CCR7-) phenotype during progression (EM CD4* = P=0.0353; EM CD8* = P=0.0023), PD1 expression was significantly increased during progression in absolute numbers and percentages (P=0.0196 and P=0.0168, respectively) as well as in the PD1+ EM subset (EM PD1*CD4*; EM PD1*CD8* = P=0.0024). Interestingly, we did not observe these changes in CLL patients that did not progress where the absolute numbers of cells expressing PD1 were either diminished or maintained during the follow-up, pointing out an important role of PD1 in regard to CLL progression. We observed that the percentage of CD8* cells expressing CD244 and CD160 were higher at the time of progression, especially for CD244 (P=0.0078). Moreover, the co-expression of these markers with PD1 was found on CD8* T cells and its percentage was increased during progression (P=0.0078). Among the differentiation subsets, the EM and CD45RA* (TEMRA) CD8* T cells expressed the highest percentages of CD244 and CD160. We did not observe changes in LAG3, TIM3 and CTLA4. T-bet and Eomes are essential to regulate T-cell differentiation and their expression has also been associated with a progenitor (T-bet+PD1+) or a terminal (Eomes+PD1+) exhausted phenotype in chronic viral infections. The percentage of CD8* T cells expressing high levels of Eomes and PD1 were significantly increased during progression (P=0.0186 and P=0.0286, respectively) whereas T-bet expression was more stable.

Summary/Conclusions: T cells from patients with progressed CLL show a more severe exhausted phenotype compared to diagnosis, which is characterized by an effector memory subset with higher expression and co-expression of PD1, CD244 and CD160, as well as higher levels of the transcription factor Eomes, indicating that the terminal exhausted phenotype (Eomes+PD1+) is predominant. These changes may contribute to the immune evasion that facilitates the progression and to the immunosuppressive scenario that dominates advanced stages of CLL. Functional assays to explain why this T cell subset is enhanced during progression are currently ongoing.

E996

EARLY SPECIFIC INCREASED EXPRESSION OF SURFACE IGM BUT NOT OF OTHER ASSOCIATED MOLECULES APPEARS TO REFLECT ANTIGEN DROUGHT AND DEMISE IN CLL PATIENTS ON IBRUTINIB THERAPY

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Background: B cell receptor (BCR) signaling through surface IgM (sIgM) is key to the survival and proliferation of normal and chronic lymphocytic leukemia (CLL) cells, and can be targeted effectively by the BTK inhibitor ibritinib. Chronic exposure of the BCR to (super)antigen leads to downmodulation of sIgM,
but not of slgD, levels and signaling capacity. This is evident in the circulating CLL B-cells which are characterized by variably reduced slgM levels/signaling. The variability influences outcome and cases with relatively higher slgM levels/signaling capacity, but not slgD, have more rapid progression, likely due to a proliferative component.

Aims: The aim of this study was to investigate the effect of ibrutinib in vivo on the TCR repertoire and function of slgM and of other surface molecules associated with the BCR complex on the circulating CLL cells of patients during the early phases of therapy (first 3 months).

Methods: Peripheral blood mononuclear cells were collected from 12 CLL patients prior to (pre-) and at 1 week, 1 month and 3 months following commencement of single agent ibrutinib therapy. Expression of BCR-complex-associated slgM, slgD, CD19 and other surface markers was assessed by flow cytometry. Signaling capacity following slgM stimulation was measured by immunoblotting. Following biotinylation of cell surface proteins, the N-glycosylation pattern of the m chain was assessed by immunoblotting as a readout of slgM expression and function. Data were obtained from all patients (REC H228/021).

Results: At week 1 of ibrutinib therapy, there was a dramatic increase in the expression of slgM on the circulating CLL cells (mean fold increase 1.6, P=0.001), while expression of slgD and CD19 remained constant. At this time-point, increased slgM expression associated with full N-glycan maturation of slgM heavy-chain, indicative of recovery from antigen engagement at tissue sites. Also, the slgM levels correlated with increased anti-igM mediated SYK phosphorylation (r=0.64, P=0.03), to indicate functionality upstream of BTK. Sequential assessment at month 1 and 3 revealed that slgM levels were similar to that observed prior to therapy, with preserved upstream signaling ability. In marked contrast, the other BCR complex-associated molecules slgD, CD19 and CD20 all reduced expression (P<0.001). Reduction of these markers was also accompanied by reduction of cell size and of other surface markers while overexpression of autophagy marker LC3B2 was documented.

Summary/Conclusions: Our data point to two major events dissociating slgM expression and function from other BCR-complex associated molecules. In the initial phase, the increased slgM expression and maturation, with no changes in other BCR-associated molecules, appears consequent to lack of antigen encounter, likely due to inhibition of chemokine-mediated entry to tissue sites. In the later phases the circulating CLL cells will suffer lack of tissue derived pro-survival stimuli. In their absence, CLL cells will reduce expression of several markers and cell size, possibly explained by autophagocytic mechanisms aiming to protect the circulating CLL cells from death unless ibrutinib therapy is withheld.

E962

TRB REPERTOIRE PROFILING OF TCL-1 TRANSGENIC MICE USING NOVEL NGS TECHNOLOGIES REVEALS OLIGOCLONAL EXPANSIONS: SIMILARITIES WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Findings from independent studies reported that the Bcr pathway and antigen stimulation occupy a central spot in the development of leukemia in the Eμ-TCL1 transgenic (tg) mouse, as in the case of chronic lymphocytic leukemia (CLL). Recently, the detailed characterization of the T-cell receptor beta chain (TRB) gene repertoire in patients with CLL revealed gene expression biases and oligoclonality. These characteristics strongly suggested that not only leukemic B cells, but also T cells are selected by antigenic elements. In this context, very little is known regarding the T cell compartment in Eμ-TCL1 mice.

Aims: Here, we sought to: (i) obtain a comprehensive view of the TRB gene repertoire in 10-12 week-old CTL-1 mice, and (ii) assess from an immunogenetic standpoint the extent of similarity between TCL-1 mice and CLL patients.

Methods: In total, we analyzed 18 samples from 16 TCL-1 mice that were categorized into 3 distinct groups, based on disease stage: (i) 6 mice with a clone size of <20% (group 1), (ii) 6 mice with a clone size of 30-55% (group 2) and (iii) 6 mice with >50% (group 3). Clone size was measured as the percentage of CD5+CD19+ B cells in the blood. Two different mice were studied longitudinally: one belonged originally to group 1 and progressed to group 2, while the other animal progressed from group 2 to 3. Five C57BL/6 wild-type (wt) mice were also analyzed as controls. TRBV-D-J rearrangements were amplified using the ImmunoSEQ® mouse T-cell receptor beta (mmTCRB) Kit from Adaptive Biotechnologies® and sequencing was performed using the MiSeq® Reagent Kit v3. Data were analyzed using the ImmunoSEQ® software.

Results: Only productive, in-frame TRBV-D-J rearrangements were included in the analysis. In total, 11 different TRBV gene segments were assessed. A total of 383,939 sequences (median: 14,239 sequences). The TRB gene repertoire was almost identical in all groups, including the wt mice. In detail, 5 different genes: TRBV13-02, TRBV19-01, TRBV03-01, TRBV13-03, TRBV05-01, TRBV02-01 accounted for almost 50% of the total repertoire. Concerning the TRBJ gene repertoire, the TRBJ02-07 gene was the most frequent gene in all groups. The analysis of the CDR3 length showed the same distribution in all groups with the mean and median CDR3 length being 12 amino acids. Expanded clones were observed in all samples with the average size of the 10 largest clones being: 9.8% for group 1, 18.3% for group 2, 12.9% for group 3 but only 0.4% for wt mice. Comparison of the TRBV repertoire in the expanded clones versus the general cohort revealed significant differences with genes TRBV12-01, TRBV12-02, TRBV16-01 and TRBV20-01 being frequent only in the former group. Shared or public clonotypes (identical CDR3 sequences) were only observed in longitudinal samples from the same mice, which also concerned some of the largest clones. Scanning the 10 largest clones of each sample for the existence of highly similar clones led to the identification of 48 clusters that contained 91/180 clonal sequences.

Summary/Conclusions: Overall, the TRB gene repertoires of TCLI mice were characterized by oligoclonal expansions that could persist over time. The TRB gene repertoire of expanded clones was more restricted than that of the general cohort, whereas comparisons between different samples revealed the existence of identical and highly similar clonotypes. These findings argue that (ongoing) selection by antigenic elements may shape the T-cell compartment in TCL-1 mice, similar to human CLL. These results further support the notion that this mouse model closely resembles CLL, at least from an immunogenetic perspective.

E998

ROLE OF THE COMBINATION MEK1/2 INHIBITOR BINIMETINIB AND AKT INHIBITOR MK2206 IN CLL

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Background: Clinical trials of ibrutinib and idelalisib demonstrate the efficacy of B-cell receptor-targeted therapies for CLL. We sought to investigate the efficacy of targeting both the BCR and the MAPK-ERK1/2 signaling pathways.

Aims: To evaluate the role of targeting the Ras-Raf-MEK1/2-ERK1/2 together with the PI3K-AKT pathways as a potential novel approach in treating chronic lymphocytic leukemia. In particular, assessing the efficacy of MEK1/2 inhibitor, binimetinib (MEK162), in combination with either a PI3K inhibitor, idelalisib or an AKT inhibitor, M2206.

Methods: All experiments conducted on primary CLL cells were co-cultured with CD40L-expressing stroma which mimics the support conferred by the tumour environment. Firstly, the effects of binimetinib in combination with MK2206 in primary CLL cells were investigated by western blotting with changes in the expression of phosphorylated and total forms of AKT, MCL-1, and ERK1/2 assessed. Expression of B-actin was used as a loading control.

Results:

Figure 1.

Effects of MK2206 are effective against DLL cells co-cultured with stromal cells in a dose-dependent manner. It was also observed that the primary CLL cells co-cultured with the CD40L-expressing stroma were significantly more sensitive to MK2206 than to idelalisib (Figure 1A). No cytotoxic effects of binimetinib
were observed while the combination with MK2206 was significantly more effective than either agent alone, suggestive of synergistic effects between the two drugs (Figure 1B). The inhibition of ERK1/2 phosphorylation following binetinib treatment led to an increase in the activity of AKT and a decrease in ERK1/2 phosphorylation. MK2206 completely abrogated the activity of AKT in the absence of binetinib and MCL-1 phosphorylation when combined with binetinib (Figure 2A). Although we observed a reduction in AKT phosphorylation following idelalisib treatment, there was no effect on the levels of AKT activity induced by binetinib (Figure 2A). We demonstrated that the inhibition of ERK1/2 and AKT by MK2206 and the inhibition of MAPK-ERK1/2 and AKT signaling may be effective at targeting the proliferative/drug-resistant compartment of CLL that resides in the tumour microenvironment.

E999

TARGETING HIF-1A AND ITS REGULATORY PATHWAYS AS A STRATEGY TO HAMPER LEUKEMIA-MICROENVIRONMENT INTERACTIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: The CXCL12/CXCR4 axis has a fundamental role in the microenvironment-mediated protection of chronic lymphocytic leukemia (CLL) cells from spontaneous and drug-induced cell death. The binding of CXCL12 with CXCR4 activates multiple intracellular pathways, including RhoA- and Ras-dependent signaling, and increases the activity of the transcription factor HIF-1α (Rigoni et al., Oncotarget 2015).

Aims: The purpose of this study was to identify new potential pharmacological targets involved in the CXCL12/CXCR4 axis in order to impair the protection exerted by SC towards spontaneous and fludarabine-induced apoptosis in CLL cells.

Methods: Peripheral blood was collected from 62 patients with CLL. In selected experiments, the M2-10B4 murine SC line and the HS-5 human SC line were used. Patient-derived bone marrow SC were generated from 12 patients with CLL. Where indicated, cell cultures were treated with recombinant CXCL12 (100 ng/ml), CXCR4 inhibitor AMD3100 (5 µg/ml), fludarabine (F-ara-A, 10 µM), simvastatin (1 µM), ERK1-2 kinase inhibitor PD98059 (10 µM), HIF-1α inhibitor BAY87-2243 (1 µM), and PI3K inhibitor idelalisib (10 µM). RhoA and Ras activities were evaluated by an ELISA based assay and by pull-down assay, respectively. ERK1-2, HIF-1α, and MCL-1 phosphorylation were measured by Western Blot.

Results: The exposure of CLL cells to recombinant CXCL12 led to the activation of RhoA- and Ras-dependent signaling, and to the downstream upregulation of HIF-1α. The CXCR4 antagonist AMD3100 completely abrogated the positive regulation exerted by both CXCL12 and SC, thus unravelling the key role of the CXCL12/CXCR4 axis in the SC-induced modulation of these signaling pathways. The inhibition of Ras and RhoA activity by simvastatin, and the inhibition of ERK1-2 and HIF-1α by PD98059 and BAY87-2243 effectively blocked the SC-induced expression and activity of HIF-1α, significantly impairing the SC-mediated protection of CLL cells, both in absence and presence of fludarabine. Similar effects were observed by targeting the PI3K/Akt pathway with idelalisib. We then investigated whether targeting RhoA- and Ras-dependent signaling could modulate HIF-1α also at the SC level. Simvastatin and BAY87-2243 effectively inhibited HIF-1α expression both in SC lines and in patient-derived SC. Moreover, simvastatin significantly reduced the secretion of CXCL12, which is a known transcriptional target of HIF-1α.

Summary: The conclusions of our data demonstrate that the targeting of HIF-1α and its regulatory pathways, both at the tumor cell and at the SC level is an appealing strategy to overcome the microenvironment-mediated protection toward spontaneous and fludarabine-induced apoptosis in CLL cells.

E1000

THE ROLE OF GENETIC-BASED PROGNOSTIC FACTORS IN PREDICTING MINIMAL RESIDUAL DISEASE NEGATIVITY IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS TREATED WITH FLUDARABINE, CYCLOPHOSPHAMIDE AND OFATUMUMAB

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Background: Chemoinmunotherapy with fludarabine, cyclophosphamide and rituximab (FCO) is the optimal front-line treatment for fit chronic lymphocytic leukemia (CLL) patients. IGHV mutations and FISH lesions are predictive markers of response and progression-free survival after FCR. Minimal residual disease (MRD) is the single best post-treatment predictor of long-term outcome after FCR, independent of biologic prognostic markers.

Aims: To explore whether conventional biologic markers (i.e. IGHV mutations, FISH lesions) and TP53, NOTCH1, BIRC3 and SF3B1 mutations can predict the attainment of a MRD negativity after first-line treatment of CLL patients with FC and ofatumumab (FCO).

Methods: Eighty young (≤65 yrs) and fit CLL patients from 15 Italian centers were enrolled in the GIMEMA LLC0911 first-line trial and treated with 6 cycles of FCO. CLL diagnosis, treatment requirement and response were defined according to the 2008 iwCLL guidelines. MRD was evaluated in responding patients by 8-color flow cytometry in the peripheral blood (PB) and bone marrow (BM) 2 months after the end of induction (month +8), and every 6 months thereafter; if negative cases were analyzed by RQ-PCR, according to the guidelines. The association between CLL biologic markers and MRD clearance after FCR was tested by Fisher’s exact test; logistic regression models were used to estimate the risk values in univariate and multivariate analyses.

Table 1.
E0101
ISOCHROMOSOME 17q, UNBALANCED TRANSLocations AND 8q GAIN REpresent ADVERSE PROGNOSTIC FACTORS IN CHRONIC LYMPHOCYtic LEUKEmIA (CLL) WITH 17p DELETION. A GfCH STUDY

Background: Chromosomal abnormalities are present in about 80% of CLL. Among them, the short arm of chromosome 17 (17p-) is the most common at diagnosis (<10%), is frequent in relapsed or refractory patients (<50%) and is associated with short survival. Loss of 17p results from various chromosomal abnormalities, including unbalanced translocations, deletions, rings or isochromosomes. All these aberrations lead to the loss of one copy of the TP53 gene, the remaining allele being generally mutated. In addition, 17p- is frequently accompanied by genomic complexity. Patients with 17p- typically progress quickly and are refractory to most conventional therapies.

Aims: We evaluated if the type of chromosomal abnormality leading to 17p- and the additional aberrations could influence the prognosis.

Materials and Methods: Data from a multicentric and retrospective cohort of 195 CLL patients harboring a TP53 deletion detected by FISH, with an informative conventional karyotype (K). All the K were reviewed by the members of the Groupe Francophone de Cytogénétique Hématologique. Overall survival (OS) and time to first treatment (TTFT) were calculated from diagnosis to death or first-line treatment, respectively, or last follow-up. The log-rank test was used to compare Kaplan-Meier curves. Cox regression models were used for multivariate analyses.

Results: The ratio H/F was 1.9. At diagnosis of CLL, the median age was 63 years [33-88]. 59% were Binet stage A, 28% B, 12% C. IGHV genes were unmutated in 40% and mutated in 60% patients tested. The IGHV genes were found mutated in 8% and unmutated in 92% of 47 patients tested. The median time of diagnosis was 13 months and a median of 2 lines of treatment [1-10]. In 28/124 (23%) cases, the 17p- was not present at diagnosis and occurred after the first therapy, with a median time of 77.5 months [22-291] from the diagnosis. Karyotype was complex in 65% of cases, and monosomal in 34% (4/12); 100% of 17p- was the consequence of an unbalanced translocation (n=167/240, 70%), with various chromosome partners, the most frequent being the recurrent der(17;18)(q10;q10) (n=32, 13%), followed by translocations involving chromosomes 8, 13, 14, 21, 15. Unbalanced translocations involving 17p and chromosome 8 (n=26, 11%), lead either to del8p (n=17), gain8q (n=6), or del8q (n=3). The other 17p abnormalities were: deletion 17p (n=45, 19%), monosomy 17 (n=15, 6%), isochromosome 17q ([i(17q)] (9, 4%) and ring of chromosome 17 (n=4, 1%). Among the additional abnormalities accompanying the 17p- unbalanced translocations were found in 121/195(63%) of patients. Combining FISH and K, del13q was detected in 71/118(60%) of cases, del8p in 40/189(21%), tr12 in 30/195(15%), gain8q in 13/105(12%), and del11q in 20/161(12%). By univariate analysis, the parameters which were associated with significantly shorter TTFT were: age ≥65 years (n=35; P=0.0038). A multivariate model including FISH lesions, gene and IGHV mutations supports the independent role of FISH and IGHV profile in predicting MRD negativity by flow and QRCR, respectively.

Summary/Conclusions: Among the high risk group of 17p- CLL, i(17q) confers a shorter OS than the other 17p abnormalities. In addition, the gain8q aggrava the outcome as well as the presence of additional unbalanced translocations. These results confirm that patients with 17p- CLL have a variable clinical course and highlight the relevance of conventional karyotyping to identify the alterations that modulate the prognosis within these patients.

E0102
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Background: Microenvironment found in bone marrow and lymph nodes supports survival, proliferation and drug resistance in chronic lymphocytic leukemia (CLL). Indeed, CLL cells are highly dependent on interactions with the microen-vironment. The BCR is one of the key players involved in the crosstalk between CLL cells and the microenvironment. Furthermore, it has a critical role in patho-genesis and prognosis of CLL. Accordingly, different factors related to increased BCR signaling are adverse prognostic factors in CLL, such as IGHV genes, high expression of ZAP-70 and increased serum levels of CCL3. Expression of ZAP-70 in CLL cells has been related to enhanced response to BCR stimu-lation, as well as to increased response to diverse stimuli that are not specific for the microenvironment. MiR-21 is an oncogenic microRNA that has been found to be overexpressed in a wide variety of neoplasms where it participates in oncogenic events such as proliferation, resistance to treatment, and metas-tasis; its overexpression in CLL has been associated to refractoriness to flu-oxazolam and to shorter overall survival and higher probability of progression.

Aims: In order to further elucidate the molecular mechanisms defining bad prognosis CLL by further elucidation of the role of ZAP-70 in the crosstalk between CLL cells and the microenvironment, we studied the relationship between ZAP-70 protein and miR-21 expression.

Methods: Peripheral blood mononuclear cells (PBMC) from 48 patients diagnosed with CLL were isolated by Ficoll-Paque Plus density gradient centrifugation. Ramos B-cells stably transfected with a vector encoding for ZAP-70 protein fused with Green fluorescent protein (GFP) or GFP only as a control were treated with Akt (LY294002), MDK (PD98059) and STAT3 (USI-124) inhibitors for 1 hour. BCR was stimulated with F(ab)2 anti-IgM. PBMC were co-cultured with bone marrow stromal cells with CD40L and CpG to mimic the microenviron-ment found in proliferation centers. After 48 hours CLL cells were harvested to analyze cell viability, cell proliferation and mRNA expression. Expression levels of ZAP-70, miR-21, PTEN, PDCD4 and PIAS3 were measured by QRT-PCR.

Results: First, we observed that miR-21 expression was significantly higher in patients with high expression of ZAP-70. Subsequently, using stably transfected Ramos B-cells with ZAP-70 protein we found that pri-miR-21 and mature miR-21 were significantly increased upon co-culture, which was enhanced by ectopic expression of ZAP-70. We also observed that inhibition of both MAPK and STAT3 pathways impairs the regulation of miR-21 expression after ZAP-70 activation. Moreover, the induction of miR-21 expression after ZAP-70 activation also induced downregulation of the tumor suppressor genes PTEn, PIAS3 and PDC4. Therefore, we postulated that the micro-environment found in primary CLL cells induced ZAP-70 and miR-21 expression, as well as downregulation of the putative miR-21 targets. Interestingly, the increase in miR-21 after co-culture was significantly impaired by ibritinib, indicating that the BCR signaling pathway is involved in its regulation in primary CLL cells. Furthermore, the miR-21 co-culture-induced increased CLL survival correlated with miR-21 upregu-lation.

Summary/Conclusions: In conclusion, stimuli from the microenvironment are capable of regulating expression of miR-21 and tumor suppressor genes.
E1003
IMPACT OF RECURRENT MUTATIONS ON PROGRESSION-FREE SURVIVAL IN CLL PATIENTS TREATED WITH FRONT LINE RITUXIMAB-BASED REGIMENS

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Background: Regimens consisting of rituximab and DNA-damaging drugs represent an important therapeutic option for patients with chronic lymphocytic leukemia (CLL). Up-to-date studies including clinical trials agreed upon the adverse outcome of TP53-defective patients that should be provided alternative treatment approaches. Additionally, mutations in NOTCH1 gene were connected with a lack of benefit from rituximab added to chemotherapy. A potential impact of other mutations commonly occurring in CLL patients remains less clear, namely regarding a role in relapse development.

Aims: (a) to assess impact of mutations in ATM, SF3B1, NOTCH1 and BIRC3 genes on progression-free survival (PFS) in CLL patients treated with front line rituximab-based regimens, and (b) to analyze clonal evolution of mutations in relapse.

Methods: We analyzed 53 CLL patients administered first line regimens FCR (fludarabine, cyclophosphamide, rituximab) or Q-FCR (FCR for reduced doses) or BR (bendamustine, rituximab); all harbored intact TP53 gene as assessed by FISH and the yeast functional analysis; 46/53 (87%) had unmaturated IGHV. The next generation sequencing using MiSeq (illumina) was done in 48 cases (38.5%) including 41 relapsed samples using three different panel: ATM (exons 2-6; median coverage (MC) 6100), SF3B11 NOTCH1/BIRC3 (exons 14-16, part of 34, and 7-10, respectively, MC 11200), and TP53 (exons 2-11; MC 31500). Functional impact of ATM mutations was verified by SIFT and PolyPhen online tools. Only mutations present in >10% of reads were considered for the PFS analysis (log-rank Mantel-Cox test); the interval was calculated from therapy completion to clinical progression (as defined by the iwCLL). Untouched peripheral blood B cells were purified using the RosetteSep isolation kit for human B cells. We characterized c-Cbl total protein level and c-Cbl(Y700) release from its autoinhibited structure by triggering a conformational change that leads to an enhanced transfer of ubiquitin from the E2 enzyme to the ligase, and a critical role has already been ascribed to B-cell receptor (BCR)-Lyn axis. We reported that in Chronic Lymphocytic Leukemia (CLL) Lyn is over-expressed and is in an active conformation as integral component of an aberrant cytosolic multisport complex, associated with several proteins, (FXR4RhoGDI, HSP90, STAT3 transcription factor). Results: We demonstrated that CXCR4 dimCD5 bright cells have higher phosphorylation of several proteins involved in Pi3K/BCR/NFkB signalling pathway (P<0.05) compared to CXCR4 brightCD5 dim cells obtained from the same patient. This lead us to hypothesize that regulation of the phospho-ST2 (a component of BCR complex that augments signal transduction) is likely of physiological importance for Pi3K/BCR signalling. Indeed, we observed significant reduction in phosphorylation of tyrosine-protein kinases associated with Pi3K/BCR signalling after silencing of CD20 by siRNA in B cells.

Summary/Conclusions: We showed that CXCR4 brightCD5 dim CLL subpopulation also have higher levels of CD19 (1.8-fold, P<0.0001), which is an important component of BCR complex that augments signal transduction. Furthermore, we demonstrated that CXCR4 dimCD5 bright cells have higher phosphorylation of several proteins involved in Pi3K/BCR/NFkB signalling pathway (P<0.05) compared to CXCR4 dimCD5 bright cells obtained from the same patient. This lead us to hypothesize that regulation of the phospho-ST2 (a component of BCR complex that augments signal transduction) is likely of physiological importance for Pi3K/BCR signalling. Indeed, we observed significant reduction in phosphorylation of tyrosine-protein kinases associated with Pi3K/BCR signalling after silencing of CD20 by siRNA in B cells.

Background: We hypothesised that the higher levels of CD20 on CLL cells that interacted with stromal cells in vivo can be characterised by relatively weak cell-surface expression of chemokine receptor CXCR4 and high expression of activation marker CD56 membrane glycoprotein. We have previously shown that BCR-Lyn axis is a critical mediator of CLL cell survival. Moreover, CLL cell clusters also have higher CD20 expression than CLL cells circulating in the peripheral blood without contact with immune niches (FXR4RhoGDI, CD56; Pavlasova et al, 2016). We hypothesised that the higher levels of CD20 on CXCR4 dimCD5 bright cells make them the primary target for RTX in vivo, since the cell killing in CD20 expression is associated with the RTX causal chemokine (Calissano et al, 2001). We analysed blood samples obtained from CLL patients treated with RTX as a single agent and indeed, we observed that RTX preferentially and nearly completely eliminates the CXCR4 dimCD5 bright subpopulation after the first RTX dose (8.3% pre-RTX vs 2.1% post-RTX; P<0.0001). We further observed a significant decrease in phospho-STAT3 expression (change that leads to an enhanced transfer of ubiquitin from the E2 enzyme to the ligase) in CXCR4 dimCD5 bright CLL cells compared to CXCR4 brightCD5 dim cells obtained from the same patient. This led us to hypothesize that regulation of the phospho-ST2 (a component of BCR complex that augments signal transduction) is likely of physiological importance for Pi3K/BCR signalling. Indeed, we observed significant reduction in phosphorylation of tyrosine-protein kinases associated with Pi3K/BCR signalling after silencing of CD20 by siRNA in B cells.
B cells we performed a co-immunoprecipitation assay, followed by Western blotting analysis, at steady state and after IgM (10µg/ml) stimulus. We also evaluated the interaction between c-Cbl and Lyn after treatment with 17-DMAG (500nM), a potent HSP90 inhibitor.

**Results:** We demonstrated that c-Cbl is overexpressed (p<0.001). Student's t test in CLL B lymphocytes with respect to normal B cells. We found that in neoplastic B cells c-Cbl did not co-immunoprecipitate with Lyn neither after BCR trigger. We obtained similar results when we treated neoplastic B lymphocytes with 17-DMAG to dissociate the Lyn-Hsp90 complex: after 1h, 2h and 4h of treatment we immunoprecipitated Lyn demonstrating that neither before nor after IgM stimulation c-Cbl interacts with this kinase. These results support the hypothesis that c-Cbl is not involved in Lyn turnover. Data obtained from 10 independent experiments showed that in CLL neoplastic cells the phosphorylation on Y700 increased after 5' and 10' of IgM stimulus, highlighting the involvement of c-Cbl in BCR signaling.

**Summary/Conclusions:** These preliminary results prompt us to investigate the role and the involvement of neoplastic clone. In CLL cells c-Cbl is overexpressed with respect to normal B cells, and upon BCR engagement it undergoes Y700 phosphorylation. However, c-Cbl is unable to stably interact with Lyn suggesting an altered c-Cbl function that contribute to affect cell homeostasis.

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**E1006**

**ACTIVATION OF SHP-1/PP2A PATHWAYS TRIGGERS APOPTOSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS**

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**Background:** CLL B cells inability to reach programmed cell death is due to intrinsic defect and extrinsic factors. Among the intrinsic fault there is the misregulation of the phosphorylation pattern. Reversible protein phosphorylation is a fundamental post-translational modification by which virtually all cellular events are regulated. The crucial players involved in this dynamic process are protein kinases and protein phosphatases, which are placed at the different levels of cellular signaling. The Src Family Kinase (SFK) Lyn is a key factor in the dysregulation of survival and apoptotic pathways of malignant B cells in CLL. One of the effects of Lyn's action is the spatial and functional segregation of the tyrosine phosphatase SHP-1 into two pools, one beneath the plasma membrane in an active state promoting pro-survival signals, the other in the cytosol in an inhibited conformation and unable to counter the elevated level of cytosolic tyrosine phosphorylation.

**Aims:** Because CLL is characterized by a high level of Lyn-dependent tyrosine phosphorylation in the cytosol, we focused our attention on compounds capable of directly or indirectly driving the activation of SHP-1 which in turn could counter the action of Lyn and induce cell demise. The goal is to discover new therapeutic strategies to defeat a still incurable disease as CLL.

**Methods:** B cells were collected from 37 CLL patients. Freshly isolated CLL cells incubated with increasing concentrations of nintedanib (0-24 µM) and MP07-66 (2,2-diethoxyethyl[[4-[(thiolyxyl)phenyl]methyl]amine) for 24 and 48 hours with/without a layer of Mesenchymal Stromal Cells (MSCs). Caspase dependence was demonstrated using the pan-caspase inhibitor z-VADfmk. CLL B cells viability was tested by Flow Cytometer with Annexin V/PI test, and with Western blotting analysis, at steady state and after IgM (10µg/ml) stimulus. We also evaluated the interaction between c-Cbl and Lyn after treatment with 17-DMAG (500nM), a potent HSP90 inhibitor.

**Results:** We performed in vitro phosphatase activity assays on the cytosolic pool of SHP-1 in the presence of increasing concentrations of nintedanib, a receptor tyrosine kinase inhibitor recently shown to trigger SHP-1 activity. Nintedanib treatment could activate the phosphatase (at Ser951), and inhibited, form of SHP-1 and to induce apoptosis, depending on the caspase activation, after 24h and 48h at marked level. Interestingly, we recently demonstrated that Ser91 phosphorylation of SHP-1 could be dephosphorylated by PP2A. In this scenario, the restoration of PP2A activity by a fingolimod-like drug devoid of immunosuppressive action, called MP07-66, and the subsequent dephosphorylation of PP2A substrates, was shown to trigger apoptosis, like nintedanib, in a caspase-dependent manner. Since our data suggest that the activation of either PP2A or SHP-1 triggered by specific small molecules caused stimulating survival or apoptotic effect in each cell, we treated CLL cells with nintedanib and MP07-66 together demonstrating an improved effect when used in combination. Similar results, in all the conditions, were obtained in presence of a MSC layer, showing the capability of these treatments to counteract the protective action of tumor microenvironment.

**Summary/Conclusions:** In conclusion, our findings indicate that phosphatase activators may represent a new weapon against this form of leukemia. Overall, these data corroborate the hypothesis that the inhibition of PP2A is central to CLL cell viability and that its activation is facilitated by the supportive action of SHP-1, as demonstrated by the effect produced by the simultaneous use of the respective activators.

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**E1007**

**TARGETING NANOPARTICLES TO CHRONIC LYMPHOCYTIC LEUKAEMIA: EXPLOITING THE PROPERTIES OF CXCR4**

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**Background:** Nanoparticle carriers of therapeutic agents (“drug delivery vehicles”) can be used to deliver drugs to specific cells through the incorporation of a “targeting ligand.” Targeting provides the therapeutic benefit of achieving high local drug concentrations while reducing off-target effects against other cells; the combined ligand/delivery vehicle system can also be manipulated to determine the uptake pathway or modulate biological effects. The CXCR4 chemokine-receptor is an attractive target for drug delivery vehicles. It is overexpressed in cancers including chronic lymphocytic leukaemia (CLL) (Domanicka et al., 2013) and binding to its ligand (CXL12) may induce proliferation, survival or entry into protective cellular niches (Ganju et al., 1998). Targeted nanoparticles that can bind and antagonise CXCR4 could therefore allow specific drug delivery to cancer cells while simultaneously blocking CXCL12-induced chemoprotection.

**Aims:** A drug-design strategy was developed to synthesise and evaluate a novel CXCR4 targeting motif (BAT1) with structural similarity to Plerixafor, a CXCR4-antagonist in clinical use. A key design principle was to incorporate a polyethylene glycol (PEG) tether with a functional end-group to provide an attachment point for cargoes, particularly liposomes. The evaluation aim was to assess the effectiveness of BAT1 to deliver a chemotherapy cargo to CLL cells within an ex vivo culture system.

**Methods:** A three-step synthesis was used to generate BAT1 (Figure 1A); its structure and purity was confirmed using NMR, MS and HPLC. Bioactivity testing employed primary CLL lymphocytes. Assays assessed: CXCR4 binding-affinity (flow cytometric competition assays), cell-binding characteristics (immunocytofluorescence and blockade of CXCL12-induced signalling (immunoblot). Initial targeting assessment used a fluorescent label (Cy5) conjugated to the functional PEG tether. Cholesterol chloroformate was then selected to conjugate BAT1 to PEGylated liposomes.

**Results:** The binding affinity of BAT1 (Figure 1B) was demonstrated using competition assays (CXCL12, anti-CXCR4 Ab, and the bis(cyclam) drug Plerixafor). The studies confirmed BAT1 had high affinity for CXCR4 receptors expressed on primary CLL cells. Immunocytofluorescence comparison with its native ligand confirmed binding of BAT1 to the CLL cell surface, while immunoblotting demonstrated blocking of CXCL12-induced signalling (Figure 1C and 1D). The fluorescent moiety Cy5 was covalently linked to the PEG moiety as a test-cargo, demonstrating that binding affinity was retained in the presence of a cargo and that the drug competed for CXCR4 binding with related bis(cyclam) drugs. This work has been extended to attach BAT1 to liposomes, with present work optimising liposome characteristics for binding and uptake by CLL and the delivery of cytotoxic payload.

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**Figure 1.**

**Summary/Conclusions:** A novel bis(cyclam) CXCR4 antagonist and targeting motif – BAT1 – has been synthesised. BAT1 demonstrates high affinity for the CXCR4 receptor, supporting targeted delivery to CLL cells. Receptor binding is associated with simultaneous blockade of CXCL12-mediated signal initiation and effect, and therefore biological modulation of target cell behaviour. BAT1
is readily attached to liposomes through the PEG moiety, which will allow chemotherapy delivery via stealth-liposomes (Allen and Cullis, 2013). Liposome size and composition will be used to drive pathway-specific uptake to different intracellular compartments. BAT1 therefore offers significant potential to enhance therapy in CLL.

**E1008**

**THE ROLE OF THROMBOPOIETIN AS A TOOL OF IMMUNE MODULATION IN CHRONIC LYMPHOCYTIC LEUKEMIA**

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**Background:** Thrombopoietin (TPO) is the major regulator of platelet production, synthesized mainly by liver cells. The TPO receptor (TPO-R) is known to be expressed on platelets, megakaryocytes and CD34+ cells. It has been reported that patients with immune thrombocytopenic purpura, treated with TPO-R agonists, developed alterations in the T-cell repertoire and pattern of cytokine secretion from B- and T-cells. Thus, clinical activity of these agents could be attributed in part to immune modulation. In chronic lymphocytic leukemia (CLL), characterized by aberrant T-cell responses, high TPO serum levels coexist with low levels of TPO gene transcripts in the malignant cells. These observations could imply that TPO acts as an immune modulator in CLL.

**Aims:** The aim of the current study was to explore the role of TPO in T-cell modulation in CLL.

**Methods:** B-cells and CD4+T-cells were isolated from peripheral blood mononuclear cells (PBMCs) of untreated CLL patients (Rai stages 0-IV) and healthy donors. CLL cells were activated with anti-CD40 antibodies to achieve adequate TPO expression. CD4+ T-cells were incubated with low-dose IL2 and TPO for 5 days and the percentage of CD4+CD25+FOXP3+ cells (Tregs) was assessed. T-cell proliferation was evaluated using CFSE staining after stimulation with anti-CD3/CD28 antibodies, high-dose IL2 and TPO for 5 days. Percentage of cells retained in G0 (non-proliferating pool) was assessed. CLL B-cells were activated with a TLR9 agonist (ODN) and TPO expression was assessed by Q-PCR.

**Results:** CD4+ T-cells of CLL patients expressed significantly higher levels of TPO-R (CD110) compared to T-cells of healthy donors, with a mean fluorescent intensity of 764±148 and 498±206, respectively (p<0.05; n=6). Stimulation of patient T-cells with TPO led to a 12% increase in the number of Treg levels. These effects have been observed in patient T-cells only, which significantly increased to 1033±342 (p<0.05; n=6) upon ODN activation.

**Summary/Conclusions:** In our study we aimed to i) detect ROR1 in CLL cells during different stages of the disease using flow cytometry and qRT-PCR with focus on patients undergoing therapy; ii) analyse changes in ROR1 expression within individual patients during the disease course.

**Methods:** CLL cohort consisted of 96 CLL patients (152 samples): 23 patients with stable disease, 16 patients with active disease prior first therapy intervention, 6 patients during first therapy, 13 patients in progression before second line treatment, 3 patients in complete remission, 10 refractory patients, 9 patients treated withibrutinib or idelalisib. TPO-R expression was examined by detection of ROR1 antigen in residual CLL cells during disease remission with the ability to distinguish malignant population from healthy B-cells. Using qRT-PCR we detected significantly higher levels of ROR1 mRNA in samples of treated patients (p<0.01). This observation was supported by analysis of ROR1 mRNA expression in five patients tested consecutively in different time-points. We detected ROR1 mRNA expression increase in disease progression before therapy and further increase during therapy administration. In case of remission induction we observed decrease of ROR1 mRNA level. In patients treated with ibrutinib or idelalisib we observed significant increase of ROR1 expression comparing untreated patients treated with different regimens.

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**E1010**

**NORMAL SERUM PROTEIN ELECTROPHORESIS IDENTIFIES AN EXCELLENT PROGNOSIS GROUP AMONG IGHV MUTATED PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA, WITH A MEDIAN TFS OVER 18 YEARS**

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**Background:** Approximately 36% of patients with chronic lymphocytic leukemia (CLL) have abnormal serum electrophoresis (SPE), either with hyperglobulinemia or with monoclonal immunoglobulin (lg) peak. In this study, we compared locally recruited patients with normal and abnormal SPE.

**Aims:** The aim was to identify prognostic parameters.

**Methods:** A total of 189 patients (132 abnormal SPE and 57 normal SPE) were included. Diagnoses were performed between 1980 and 2015. Prognosis parameters investigated were IGHV mutation status, presence of SF3B1, NOTCH1 or BIRC3 mutations (determined by high throughput and Sanger sequencing), and cytogenetic abnormalities such as del17p, del11q, del13q and trisomy 12 (assessed by standard cytotype, FISH analysis and QMPSF).

**Results:** In this series, 73%, 19% and 8% of patients were at Binet stage A, B and C respectively, and 30% had a normal SPE at diagnosis. Ninety six percent of patients with normal SPE were at Binet stage A, versus 63% of patients with abnormal SPE (Chi2 test : p<10^-5). Median lymphocytosis at diagnosis was lower in patients with normal SPE (12.82 G/L versus 19.54 G/L in abnormal SPE; Chi2 test : p=0.019). Referring to three prognostic markers, we found that 58% of cases with normal SPE had a good prognosis profile (mutated IGHV and/or isolated del13q, with no other genetic abnormality detected), meanwhile 65,2% of patients with abnormal SPE exhibited at least one poor prognosis marker (unmutated IGHV, mutation of SF3B1, NOTCH1, del17p, or trisomy 12). Chi2 test : p<10^-4). In patients with normal SPE, only 3,5% cases were SF3B1 mutated against 15,2% in case of abnormal SPE (Chi2 test : p=0.002). Among other strong differences, 10,5% patients with normal SPE had a trisomy 12 against 18,2% for abnormal SPE. Isolated del13q was found in 38,6% and 21,2% of cases with normal and abnormal SPE respectively. Mutated IGHV status in FCR was found in 65% in normo with a 56% with abnormal SPE. Compared to the whole series, IGHV repertoire analysis shows bias in IGHV1-2, and IGHV4-34 rearrangements, with decreases usage of IGHV3-21 and IGHV3-48.

**Treatment free survival was markedly increased in patients with normal SPE (median of 10.0 years versus 3.0 years for normal and abnormal SPE respectively).** Treatment free survival was markedly increased in patients with normal SPE compared to patients with SF3B1 mutated, del13q and trisomy 12. Median of 8.0 years for patients with normal SPE and 2.8 years for SF3B1 mutated. Median of 8.0 years for patients with normal SPE compared to patients with SF3B1 mutated, del13q and trisomy 12. Median TFS over 18 years. Patients with mutated IGHV and abnormal SPE had a
median TFS of 4 years (log rank test: p=0.0003). Thus, patients with normal SPE and IGHV mutated status constitute a group with excellent prognosis.

Summary/Conclusions: In conclusion, normal SPE was associated with good outcome with decreased accumulation of side genetic events (in particular, SF3B1 mutations). This analysis shows a bias in IGHV repertoire according to SPE status. These results also clearly suggest that patients with a normal SPE and mutated IGHV have an extremely quiet CLL natural history. This could be either due to the weaker activity of the disease and/or to the absence of adverse consequences of a concomitant paraprotein.

E1011
HSP70 EXPRESSION IS MODULATED BY ITS MASTER REGULATOR HSF1 VIA PI3K AND PI3K/AKT/MTOR PATHWAYS IN CHRONIC LYMPHOCYTIC LEUKEMIA

Background: The search for molecules involved in apoptosis resistance/ increased survival of B cells from Chronic Lymphocytic Leukemia (CLL) is still ongoing since this disease remains not definitively understood. We recently found that the Heat Shock Protein of 70kDa (HSP70), expressed in response to a wide variety of stress signals and allowing cells to survive to lethal conditions, was particularly overexpressed in neoplastic B cells from CLL. Moreover, the Heat Shock Factor 1 (HSF1), the major responsible for the transcription of HSP70, is itself overexpressed in CLL and strictly correlated to HSP70. In response to stress, HSF1 becomes phosphorylated, forms homotrimers, binds DNA and activates heat shock gene transcription. HSF1 is regulated by a fine balance of activatory/inhibitory phosphorylations mediated by kinases belonging to pathways triggered by RAS (i.e. PI3K/AKT/mTOR and RAF/MEK/ERK).

Aims: Since HSP70 is overexpressed in CLL neoplastic B cells and most of the "HSF1-phosphorylating actors" belong to signalling pathways taking part from RAS, being the PI3K/AKT/MTOR and the RAF/MEK/ERK pathways, we are herein aimed at gaining information and dissecting this network in CLL B cells.

Methods: In a Reverse Phase Protein Array (RPPA) study, previously performed from 57 CLL patients and 11 healthy volunteers, we evaluate the activation/expression of key signalling proteins. Herein, we focused on HSP70, AKT-Ser473, mTOR-Ser244, GSK3α/b-Ser21/9, CDK2, CREB-Ser133, Ser217/221 and ERK-Thr202/Tyr204, known to negatively regulate HSF1. The activation/expression of key signalling proteins. Herein, we focused on HSP70, AKT-Ser473, mTOR-Ser244, GSK3α/b-Ser21/9, CDK2, CREB-Ser133, MEK1/2-Tyr217-221, ERK1/2-Thr202/Tyr204, NFkB-Ser536, p38MAPK-Thr180/Tyr182, SAPK-JNK-Thr183/Tyr185 and PDK1-Ser241. Cluster and separated analyses have been performed.

Results: We divided our patients in HSP70-high and HSP70-low considering as cut-off the value of the median of HSP70 expression levels calculated by RPPA and demonstrated that the examined proteins behave in a different way between patients expressing high or low levels of HSP70. HSP70-high patients present high Akt-Ser473, an inhibitor of GSK3α/b that, in the inhibited form, prevents HSF1 inhibition. By contrast, HSP70-low patients have high MEK1/2-Ser217/221 and ERK-Thr202/Tyr204, known to negatively regulate HSF1. Intriguingly, p38MAPK-Thr180/Tyr182 which has been described to both activate and inhibit HSF1 at different sites, is overexpressed in those patients presenting low levels of HSP70.

Summary/Conclusions: These data would suggest that, in CLL, HSP70 expression is regulated by the modulation of HSF1 activity through the activation of one or the other way triggered by RAS. In particular, an activation of the PI3K/AKT/MTOR pathway leads, as result, to a higher expression of HSP70 while an activation of the RAF/MEK/ERK signalling rather results in HSP70 down regulation. The dissection of signalling pathways connected to HSP70-HSF1 axis in CLL will contribute to define the biology and understand the pathogenesis of this disease.

E1012
THE INTERPLAY BETWEEN TH17 AND TREGS: A NEW IMMUNOSUPPRESSIVE INSIGHT IN CHRONIC LYMPHOCYTIC LEUKEMIA

Background: Chronic lymphocytic leukemia (CLL) is the most frequent leukemia in the Western world and it is characterized by the clonal expansion of CD5 positive B cells. In CLL, different T cell dysfunctions have been described, probably related to the interaction with malignant B cells. TH17 and regulatory T cells (Tregs) are subpopulations of T lymphocytes which play a fundamental part in inflammatory response and immune tolerance. However, their role in the immunopathogenesis of CLL has not yet been fully clarified.

Aims: The aim of this study is to clarify the interplay between TH17 and Tregs in the pathogenesis of CLL.

Methods: After obtaining the patient’s informed consent, peripheral blood was collected from 30 untreated CLL patients and 30 age-matched healthy volunteers (HV). Cytokine production was evaluated before and after a 48 h culture of CD4+ T cells in complete medium with IL-6 (o/n), followed by a 5 h stimulation with PMA, Ionomycin and Monensin (PIM), or with Candida Albicans. For cytokine secretion analysis, stimulated CD4+ T cells were analyzed by ELISA. For the analysis of Tregs and their subsets, stimulated PBMCs were stained with anti-CD4 FITC, anti-CD25 APC-Cy7, anti-FoxP3 APC and anti-CD45RO PE or anti-CD127 PE or anti-GATA-3 PE. Statistical analysis were carried out using the paired and unpaired two-tailed Student’s tests and confirmed with the non-parametric Wilcoxon signed-rank test.

Results: In CLL patients we observed a reduced production of IFN-y and IL-4, respectively from TH1 and TH2 and an increase of IL-17A from TH17, compared to HV. All the observed differences were statistically significant. We also evaluated the ability of CD4+ T cells to secrete IL-17A, IL-10 or both. We reported a statistically significant increase in the frequency of CD4+ IL-17A-producing cells in CLL patients compared to HV, whereas the percentage of IL-17A+IL-10+ cells remained unchanged. In order to evaluate the functional effects of the observed alterations, we analyzed IFN-y+ CD4+ T cells-mediated response after stimulation with C. Albicans for 48 h, with or without depletion of IL-17A-secreting cells. The frequency of IFN-y-producing T cells resulted significantly increased in patients than HV before IL-17A-secreting T cells depletion. Conversely, after IL-17A+ CD4+ T cells depletion, we didn’t observe significant differences in term of IFN-y production. We also observed increased IL-23 plasma levels in patients compared to HV. In addition our data highlighted a significantly higher frequency of CD4+ CD25hiFoxP3+ Tregs (Tregs) in CLL samples, with a statistically significant increase in Tbet+ Tregs, RORγt+ Tregs and GATA-3+ Tregs subpopulations (Figure 1).

Figure 1.

Summary/Conclusions: Our results reported a down-regulation of IFN-y and IL-4 producing T cells, associated to an increased frequency of Tregs and their subsets in CLL patients, probably trying to overcome the deficit of effector T cells. On the other hand, we observed a rise in IL-17A secreting T cells related to the increased IL-23 production by dendritic cells in order to restore TH17 pool, without changing the percentage of IL-17A+IL-10+ cells. These data support the idea of the protective function of TH17 that show an effector and not a regulatory T phenotype. Starting from these observations, this study could pave the way to further researches and applications in the comprehension of the biological and regulatory mechanisms of TH17 and Tregs, supporting the study of a pioneering anticancer therapy in CLL.

E1013
LOW EXPRESSION OF CD25 IN CHRONIC LYMPHOCYTIC LEUKEMIA WITH NOTCH1 MUTATED CASES INDEPENDENT OF CD64/6 MISREGULATION

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Background: Recently, it has been shown that CD64-mediated repression of CD25 is required for induction and maintenance of NOTCH1-induced T-cell acute lymphoblastic leukemia.

Aims: The aim of this study was to identify the NOTCH1 mutual status detected by deep sequencing in a cohort of 138 patients, and to correlate it with the immunophenotypic profile and CD4 and CD64 expression.

Methods: We performed targeted NGS sequencing of blood samples, collected at diagnosis, from 138 CLL patients. We designed a TruSeq Custom Amplicon
containing 13 genes and covering 28,099 bases. Pair-end sequencing was performed with MiSeq v2.2 chemistry, and a mean depth of 998 reads/base was obtained. Every patient underwent, at baseline, a flow cytometry characterization with a panel including 15 antigens. The results were validated by RT-qPCR.

**Results:** With a median age of 66 y.o. (range, 31-89) and a slight male predominance, the median follow up time of our cohort was 43 months (24-104). We found that 38/138 (28%) patients harbored at least one mutation, with NOTCH1 (n=16, 12%), ATM (n=12, 9%), TP53 (n=9, 7%), and SF3B1 (n=8, 6%) being the most frequently mutated genes. These patients with one mutation showed a lower CD25 expression (24 mean fluorescence intensity units [MFU]) than those without a mutation (43 MFU), p=0.03. We could not validate the recently reported association between the presence of NOTCH1 mutations and a low expression of CD20. In our cohort, the MFI expression in NOTCH1 mutated and non-mutated patients was 163 and 146 units, respectively (p>0.05). We measured CDK4 and CDK6 expression in the CD19+ sorted fraction RNA of 7 NOTCH1 mutated cases and 11 non mutated cases, without finding significant differences (0.26 vs 0.27 for CDK6, 0.025 vs 0.022 for CDK4; p>0.5 in both cases).

**Summary/Conclusions:** We found a significant inferior expression of CD25 when activating NOTCH1 mutations are present in CLL patients. The relationship found between these two variables, with an inverted direction to that found in physiological conditions, has also been shown in the setting of NOTCH1-mutated T acute lymphoblastic leukemia. In CLL cases, it seems to be independent of CDK4/6 expression, prompting further studies to consider CDK4 and CDK6 regulators.

**E1014**

**GENE MUTATIONS ANALYZED BY NEXT-GENERATION SEQUENCING ALLOW US TO DEFINE THE PROGNOSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS WITH EARLY-STAGE DISEASE AND 13Q DELETION**

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**Background:** Next-Generation sequencing (NGS) studies have revealed a number of recurrently mutated genes in chronic lymphocytic leukemia (CLL). It is reasonable to argue that evaluation of the newly gene mutations as prognostic markers would help to improve prognostication of CLL patients. Interestingly, gene mutations could help us to refine the prognosis in the group of CLL patients with other prognostic markers associated with good prognosis.

**Aims:** To analyze the presence of mutations of a panel of genes by NGS and its prognostic impact in patients with CLL, focusing in the groups of patients with good prognosis characteristics.

**Methods:** Amplicon-based NGS was performed using 454 platform in 147 CLL patients to evaluate the mutational status of genes (TP53, NOTCH1, SF3B1, XPO1, FBXW7 and MYD88). Samples were obtained at diagnosis or before treatment in all cases. 70.1% were Binet A and 53% had 13q deletion (13q-). A cut-off 2% was applied to define variants. All the mutations were validated.

**Results:** 1. NGS analysis showed that 37.4% of CLL patients (55/147) showed mutations in any of the analyzed genes. The frequency of mutations was 16.3% for NOTCH1, 10.2% for SF3B1, 6.8% for TP53, 4.8% for XPO1, 3.4% for FBXW7, and 1.4% for MYD88. The presence of mutations in any of these genes except to MYD88 (mutated CLL) was significantly associated with clinical progression (60.0% for mutated CLL vs. 38.2% for unmutated CLL; P<0.05). Interestingly, mutated CLL patients showed a shorter time to first treatment (TFT) than unmutated CLL patients (30 months vs. 88 months; P=0.006). By contrast, MYD88 mutations were detected in CLL with mutated IGHV and 13q-. Of note, 23.6% of the mutations had a mutational load of ≤15% and thus would not have been detected by capillary Sanger sequencing. CLLs with mutations in MYD88 showed a shorter TFT than those without mutations (18 vs. 88 months; P=0.018), and similar to CLL patients with mutations in >15% of cells (P=0.370). In addition, 14.5% of mutated CLL patients showed 2mutations.

Patients with more than one mutation had a shorter TFT than CLL patients with one mutation (7 months vs 31 months). 3. In the group of CLL patients with 13q-, 32.8% of them showed mutations in any of the analyzed genes. Interestingly, CLL patients Binet A with mutations (except to MYD88) showed a shorter TFT than CLL patients without mutations (31 vs 131 months, P<0.001). Besides this, CLL with 13q- as the sole cytogenetic alteration and gene mutations had also a shorter TFT that unmuted 13q- CLL patients (P<0.001).

**Summary/Conclusions:** 1) CLL patients with mutations in TP53, NOTCH1, SF3B1, XPO1 and FBXW7 show a worse prognosis than CLL patients without mutations. 2) Gene mutations in TP53, NOTCH1, SF3B1, XPO1 and FBXW7 in a low percentage of the cells are associated with a shorter TFT. 3) Among CLL patients with good prognostic characteristics (Binet A and 13q-), gene mutations help us to define the prognosis of the patients.

**E1015**

**ALTERED COMPLEX C5 IS ASSOCIATED WITH COMPROMISED COMPLEMENT ACTIVITY IN CHRONIC LYMPHOCYTIC LEUKEMIA**

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**Background:** The therapeutic monoclonal antibodies used for the treatment of Chronic lymphocytic leukemia (CLL) mediate anti-tumor effects through several independent cell-mediated cytotoxicity (ADCC), and phagocytosis. CDC efficacy thus depends on the expression level of the target B-cell antigen, the integrity of apoptotic cascades within tumor cells, the functional capacity of effector cells, and the availability and activity of the complement (C) system. Published data indicate deficiency of one C protein or more in most CLL patients, as well as additional factors that may affect C activity. The role of structural abnormalities of C complexes in affecting C function has not been investigated.

**Aims:** To study the structural integrity of circulating C complexes, focusing mainly on C5, and to establish its importance for C activity in CLL.

**Methods:** Blood samples were obtained from 35 (24 Binet A and 10 healthy controls (HC). Biochemical and haematological parameters, and C5 staging were recorded. The isoforms of two C components, C5 and C5b, were studied by Western blot analysis. The activity of the C system before and after in-vitro activation via the classical or alternative pathways was followed by the levels of C5b-9, the terminal product of C activation. C activation was also studied in vitro and in-vitro activation data were associated with the presence of C5 isoforms.

**Results:** C5 isoform differences in C5 pattern were found in some of the CLL patients. Specifically, the C5 complex that exists as a single protein band in all HC and in 56% of CLL patients appeared in 44% of the patients as a clear double band. No clear differences were observed in C3 pattern in the patients. Higher basal levels of C5b-9 were found in CLL patients with abnormal pattern of C5 (3946±758 SEM ng/ml) compared with both HC (994±158 SEM ng/ml) and CLL patients presenting normal C5 pattern (2363±655 SEM ng/ml). In-vitro C activation via the classical pathway was inversely correlated with basal activity, and was significantly lower (p<0.03) in the CLL patients with altered C5 compared to HC and CLL patients with normal C5. In-vitro activation via the alternative pathway was similar in all subjects' groups. C activity in C5-deficient serum supplemented with 33% sera from patients with abnormal C5 was significantly lower compared to the activity observed after supplementation with serum from HC or from patients with normal C5. Activity after supplementation with normal C5 (commercial) was significantly lower in sera from CLL patients with abnormal C5 than sera from patients with normal C5. The exact mechanisms by which abnormal C5 distracts the C activity need further clarification. Yet, the appearance of abnormal C5 in CLL patients with disturbed C activity bears the potential to develop a marker which will assist in identifying patients who are likely to be less responsive to future immunotherapy treatment due to compromised CDC. Development of such a marker may assist clinicians in refining and personalizing the immunotherapeutic approach, improving CDC and consequently the therapy results.
Chronic lymphytic leukemia and related disorders - Clinical

E1016
ASSOCIATION OF CGP-STIMULATED KARYOTYPE WITH TIME-TO-FIRST TREATMENT FOR CLL
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Background: Prognostic factors correlate with clinical outcomes, independent of treatment. B cell receptor (BCR) signaling pathway inhibitors can nullify the prognostic impact of some markers, such as IGHV mutation status. CpG-stimulated metaphase karyotype can identify clonal cytogenetic abnormalities in CLL that may not be seen with standard non-stimulated karyotype or by FISH. Complex cytogenetics, defined as 3 or more chromosome abnormalities in 2 or more metaphases was the highest-risk feature for shorter progression-free and overall survival in patients receiving ibrutinib for relapsed/refractory CLL. Complex karyotype is not uncommon among relapsed/refractory CLL cases, particularly those who previously received genotoxic chemotherapy.

Table 1. Continuous and Categorical Patients Characteristics.

Aims: We performed a comparison of the CLL-IPI with the Barcelona-Brno prognostic model in an independent series of Italian and United States (U.S.) patients.

Methods: Databases from 4 Italian and 1 U.S. centers including roughly 3700 newly diagnosed CLL patients were used to compare the CLL-IPI with the Barcelona-Brno prognostic model. Baseline data regarding age, Rai stage, IGHV mutational status, β2M and fluorescence in situ hybridization (FISH)-detected cytogenetic abnormalities were available for 1299 cases. Del17p was used as the sole marker of TP53 status. The CLL-IPI and the Barcelona-Brno prognostic model were calculated using the methods proposed. The accuracy of the prognostic models was assessed by the Harrell C index (an index of discrimination), and the Akaike information criterion (AIC, an index comparing two non-nested prognostic models). The lower the AIC, the higher the prognostic accuracy of a predictive model.

Results: The median age of the 1299 patients was 63 years (range 27-92) with 61.3% males. The majority of patients had Rai stage 0 (57.9%). According to the CLL-IPI, 51.3% of patients were classified as low-, 28.7% as intermediate-, and 10.1% as high-risk. The majority of patients were classified as low-, 31.8% as intermediate-, and 10.1% as high-risk. The proportion of patients with del17p was 18.1%.

Discussion: These results confirm the potential utility of a biologically driven risk model in predicting OS and TTF in patients with CLL. The Barcelona-Brno model showed a better performance compared to the CLL-IPI, with 61.3% of patients being classified as low-, 31.8% as intermediate-, and 10.1% as high-risk. The proportion of patients with del17p was 18.1%.

In conclusion, CpG-stimulated karyotype identified 1 or more clonal chromosome abnormalities in nearly a third of untreated patients and was a significant independent prognostic factor for TTF. Models for TTF may be useful in identifying patients at high-risk for needing treatment sooner and thereby useful for early intervention clinical trials.

haematologica | 2017; 102(s2) | 417
Background: BCL-2 is an anti-apoptotic protein overexpressed in chronic lymphocytic leukemia (CLL). BCL-2 is responsible for apoptosis machinery dysregulation and contributes to chemotherapy resistance. S55746/BCL201 is a novel, oral, selective BCL-2 inhibitor.

Aims: The current first-in-human study evaluates the safety and aims to establish the recommended phase 2 dose; main secondary objectives include evaluation of pharmacokinetics (PK), food effect, pharmacodynamics and preliminary activity in patients (pts) with relapsed or refractory CLL.

Methods: S55746/BCL201 as single agent is being investigated in a phase I (EUDRACT, NCT02920697), open-label, multinational, international dose escalation trial. S55746/BCL201 was initially administered in fasting condition, once daily (in 21-day cycle) until progressive disease (PD) or unacceptable toxicity. A tumor lysis syndrome (TLS) prevention protocol was implemented. After giving informed consent pts could receive 50 to 2000mg according to a modified version of the Continual Reassessment Method for dose allocation process. In the food effect part of the study, 7 non-Hodgkin’s lymphoma patients received a film coated 200mg tablet under fasting condition (i.e. after 10-hour fasting period) and after a calibrated moderate meal the day after.

Results: As of February 2017, 12 CLL pts have been treated (median age 67 years [range 52-82]). On these 12 pts, 5 presented a bulky disease, 1 a 17p deletion, 4 a 11q deletion, and 1 a p53 mutation. CLL pts were dosed up to 700mg, with a median duration on treatment of 79 days. Median number of prior regimens in CLL pts was 4 (range 2-5). Preliminary PK results in fasting pts showed that exposure increased linearly but with some inter-individual variability. The most frequent (≥2 pts) grade 3/4 adverse events (AEs) were neutropenia (n=2) and thrombocytopenia (n=2). AEs possibly related to the study drug were reported in 4 pts: neutropenia (n=2), neutrophil count decrease (n=1), fatigue (n=1), dyspnea (n=1), gingival bleeding (n=1) and left ventricular ejection fraction (LVEF) decrease (n=1). No clinical or laboratory TLS were reported. One patient in the 700mg cohort experienced a DLT (asymptomatic LVEF decrease grade 2 recovered within 2 weeks). At 700mg, a decrease in lymphocytes count from baseline (≥50%) was observed in 3 out of 4 patients associated with a decrease in the sum of the product of the diameters of lymph nodes (from 23% to 40%). This decrease in lymphocytes count, started from cycle 1, and may be correlated with an induction of apoptosis in CLL cells (4 hours post first dose), detected by flow cytometry in CD19+Annexin+ cells. Two CLL pts are ongoing after having completed their 3rd cycle; 10 pts have withdrawn from the study: 7 due to PD, 1 for lack of efficacy and 2 due to AE. The non-compartmental pharmacokinetic analysis of the food effect cohort (5 assessable pts) demonstrated that S55746/BCL201 PK is modified by the ingestion of a moderate meal (400-500 kcal with fat contributing to 150 kcal). The median Tmax was delayed from 1.5h to 4h when administered with food. Compared with fasting condition, Cmax and AUC increased by approximately 6-fold following a moderate meal. Based on these results, a protocol amendment to the clinical trial has been implemented in order to further investigate the administration with food in a new dose escalation.

Summary/Conclusions: S55746/BCL201 monotherapy showed first signs of activity across the tested dose levels with an acceptable safety profile so far. Based on PK food interaction results, dose escalation in the fed state has started.

E1018
PRELIMINARY RESULTS OF S55746/BCL201 (A NEW BCL2 INHIBITOR) IN RELAPSED OR REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS AND EFFECT OF CALIBRATED MODERATE MEAL ON THE PHARMACOKINETICS

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Methods: S55746/BCL201 as single agent is being investigated in a phase I (EUDRACT, NCT02920697), open-label, multinational, international dose escalation trial. S55746/BCL201 was initially administered in fasting condition, once daily (in 21-day cycle) until progressive disease (PD) or unacceptable toxicity. A tumor lysis syndrome (TLS) prevention protocol was implemented. After giving informed consent pts could receive 50 to 2000mg according to a modified version of the Continual Reassessment Method for dose allocation process. In the food effect part of the study, 7 non-Hodgkin’s lymphoma patients received a film coated 200mg tablet under fasting condition (i.e. after 10-hour fasting period) and after a calibrated moderate meal the day after.

Results: As of February 2017, 12 CLL pts have been treated (median age 67 years [range 52-82]). On these 12 pts, 5 presented a bulky disease, 1 a 17p deletion, 4 a 11q deletion, and 1 a p53 mutation. CLL pts were dosed up to 700mg, with a median duration on treatment of 79 days. Median number of prior regimens in CLL pts was 4 (range 2-5). Preliminary PK results in fasting pts showed that exposure increased linearly but with some inter-individual variability. The most frequent (≥2 pts) grade 3/4 adverse events (AEs) were neutropenia (n=2) and thrombocytopenia (n=2). AEs possibly related to the study drug were reported in 4 pts: neutropenia (n=2), neutrophil count decrease (n=1), fatigue (n=1), dyspnea (n=1), gingival bleeding (n=1) and left ventricular ejection fraction (LVEF) decrease (n=1). No clinical or laboratory TLS were reported. One patient in the 700mg cohort experienced a DLT (asymptomatic LVEF decrease grade 2 recovered within 2 weeks). At 700mg, a decrease in lymphocytes count from baseline (≥50%) was observed in 3 out of 4 patients associated with a decrease in the sum of the product of the diameters of lymph nodes (from 23% to 40%). This decrease in lymphocytes count, started from cycle 1, and may be correlated with an induction of apoptosis in CLL cells (4 hours post first dose), detected by flow cytometry in CD19+Annexin+ cells. Two CLL pts are ongoing after having completed their 3rd cycle; 10 pts have withdrawn from the study: 7 due to PD, 1 for lack of efficacy and 2 due to AE. The non-compartmental pharmacokinetic analysis of the food effect cohort (5 assessable pts) demonstrated that S55746/BCL201 PK is modified by the ingestion of a moderate meal (400-500 kcal with fat contributing to 150 kcal). The median Tmax was delayed from 1.5h to 4h when administered with food. Compared with fasting condition, Cmax and AUC increased by approximately 6-fold following a moderate meal. Based on these results, a protocol amendment to the clinical trial has been implemented in order to further investigate the administration with food in a new dose escalation.

Summary/Conclusions: S55746/BCL201 monotherapy showed first signs of activity across the tested dose levels with an acceptable safety profile so far. Based on PK food interaction results, dose escalation in the fed state has started.

E1019
INCREASED VIRUS-SPECIFIC IMMUNE RESPONSES PARALLELED BY A PNEUMOCOCCUS-SPECIFIC-IMMUNODEFICIENCY STATE AND HYPOGAMMAGLOBULINEMIA: ALREADY EMERGE IN HIGH-COUNT MONOCLONAL B LYMPHOCYTOSIS PRIOR TO CLL

Background: Monoclonal B lymphocytosis (MBL) display a high incidence of infections, due to an associated immunodeficiency state that includes hypogammaglobulinemia. Even more, it has been recently shown that the earlier stages of disease, i.e. high-count monoclonal B lymphocytosis (MBL), subjects also have increased risk for infection.

Aims: To evaluate the status of the humoral immune response in CLL at different disease stages, as well as in pre-leukemic MBL, and MBL low count (MBL) cases, vs healthy controls, through quantitation of soluble plasma levels of specific antibodies against ubiquitous and pulmonary infection-associated pathogens.

Methods: A total of 249 subjects (119 males/130 females; aged 68±11y) including 91 healthy donors, 71 CLL-like MBL, 29 CLL-like MBL, 58 CLL cases and 32 Binet A, and 26 Binet B/C patients were studied. Detection of clonal CLL-like B cells was performed by high-sensitive 8-color flow cytometry. Quantification of plasma antibody isotypes and specific immunoglobulins against CMV (cytomegalovirus), EBV (Epstein Barr Virus), influenza virus and S. pneumoniae were performed by nephelometry and commercial ELISA kits, respectively. Individuals who had received vaccination against Influenza and/or Pneumococcus were excluded from the analysis of the immunoglobulin-specific titers against the corresponding pathogen, respectively. Plasma CMV and EBV DNA load were assessed by real time PCR in all cases.

Results: Total immunoglobulin (Ig) titers tended to decrease with disease progression, independently of the isotype. In contrast, specific IgM and IgG titers against CMV, EBV and influenza virus did not vary among groups, with the
exception of VCA-EBV IgG titers, that were higher in CLL vs the other groups. Strikingly, the IgG levels for the three viruses tended to gradually increase, from healthy individuals to stage B/C CLL. These findings were more pronounced (p<0.05) for IgG and to a lesser extend also for IgM, when the ratios between the virus-specific IgG/total IgG titers of the same isotype were calculated, except for Influenza-specific IgG, that showed the same trend but without statistical significance. Repeating CMV DNA load, only 3177 individuals -1 MBL and 2 CLL were found to be positive (below the limit of quantitation), while EBV DNA load was detected in plasma from 7191 (all being Binet A CLL) at median levels of 3.6 copies/ul. In contrast to the virus-specific Igs, IgG plasma levels against S.pneumoniae progressively diminished through progression of the disease and were inversely related to the overall lower immunoglobulin levels.

**Summary/Conclusions:** Both MBL and CLL patients present relatively high levels of specific Ig against human host viruses in parallel to progressively lower levels of anti-S.pneumoniae antibodies, which might reflect (asymptomatic) chronic reactivation of humoral immune responses against host viruses and, consequently, progressively decreased protection against other microorganisms, denoting a severe pathogen-specific humoral immunodeficiency state not reflected by the overall plasma immunoglobulin levels. Alternatively, these results might point out a potential role of ubiquitous viruses in the pathogenesis of the disease. Further analyses are necessary to establish the potential relevance of such asymptomatic humoral immune responses against host viruses in the expansion of the tumor B-cell clone and progression from MBL to CLL.

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**E1020**

**AN EXTENSIVE MOLECULAR CYTOGENETIC CHARACTERIZATION IN HIGH-RISK CHRONIC LYMPHOCYTIC LEUKEMIA IDENTIFIES KARYOTYPE ABERRATIONS AND TP53 DISRUPTION AS PREDICTORS OF OUTCOME AND CHEMOREFRACTORINESS**

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**Background:** Chronic lymphocytic leukemia (CLL) is a heterogeneous disease, running an indolent course in some patients and a clinically aggressive course in others. Risk assessment is important in clinical practice and prediction of outcome and response to treatment is very useful in an era in which several chemomunotherapy combinations and effective mechanism-driven treatments are available.

**Aims:** We investigated whether an extended genetic characterization including mutational screening by next generation sequencing (NGS) and karyotype analysis could allow for a refinement of our capability to predict outcome in newly diagnosed CLL patients with high-risk features, as defined by the presence of unmutated IGHV gene and/or 11q22/13p13 deletion by FISH and/or TP53 mutations.

**Methods:** 101 patients were included in this study. TP53 disruption was defined by the presence of 17p13 deletion by FISH and/or TP53 mutation by NGS. Cytogenetic analysis was performed using Cpg-oiligonucleotide DSP30. Each patient was subsequently categorized according to the following classification: favorable group (isolated 13q14 deletion or normal karyotype), unfavorable group (deletions of 11q22 or 13p13, or complex karyotype, ie, at least 3 chromosome aberrations); intermediate group (all other karyotypic abnormalities). A cut-off of 98% homology to the germline sequence to discriminate between IGHV mutated and unmutated cases. Mutational screening was performed with Ion Torrent PGM NGS platform on 20 CLL-related genes by using a 5% cut off.

**Results:** Cytogenetic analysis showed favorable findings in 30 patients, unfavorable in 34 cases and intermediate in 36 cases. A complex karyotype was present in 21 patients. By NGS, 95 somatic mutations were observed in 56/101 (55.4%) cases; 80 nonsense mutations, 5 nonsense mutations and 10 frameshift deletions. 16 cases (15.8%) showed mutations in the TP53 gene, 11 (10.9%) in the NOTCH1 gene, 11 (10.9%) in the SF3B1 gene, 8 (7.9%) in the ATM gene, 5 (4.9%) in the BIRC3 gene, 5 (4.9%) in the PTEN gene, 4 (4.0%) in the MYD88 gene, 4 (4.0%) in the BRAF gene, 4 (4.0%) in the POT1 gene, and 18 (17.8%) cases in the remaining 11 genes. 26/56 (46.4%) mutated patients presented two or more mutations. The presence of mutations was associated with unmutated IGHV status (p=0.040) and the complex karyotype (p=0.047). TP53 disruption correlated with the presence of ≥2 mutations by NGS (p=0.001) and a complex karyotype (p=0.012). By multivariate analysis an advanced Binet stage (p=0.001) and an unfavorable karyotype (p≤0.01) predicted for a shorter time to first treatment (TTFT), while TP53 disruption (p=0.019) and unfavorable karyotype (p=0.028) predicted for a worse overall survival (OS). A shorter time to chemorefractoriness (TTFR) was associated with TP53 disruption (p=0.001) and unfavorable karyotype (p=0.025). Patients with both unfavorable karyotype and TP53 disruption presented a dismal outcome (median OS and TTFR of 28.7 and 15.0 months respectively).

**Summary/Conclusions:** A comprehensive analysis of chromosomal aberrations and gene somatic mutations in high-risk CLL showed that the cytogenetic profile was independently associated with a shorter TTFT, OS and TTFR. Since karyotyping using novel methodologies may contribute to the refinement of prognosis in high-risk CLL patients, the introduction of this technique in future CLL trials seems warranted to identify those patients that could be ideal candidates for consolidation treatment or novel treatment combinations.

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**E1021**

**SHOULD CLL-IPI BE USED TO ASSESS OVERALL SURVIVAL OF EVERY CLL PATIENT? A SYSTEMATIC REVIEW AND META-ANALYSIS**

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**Background:** A weighted grading approach based on five independent prognostic factors (i.e., TP53 status, IGHV mutational status, S2-microglobulin, clinical stage and age) has been used by an international Working Group to generate the chronic lymphocytic leukemia international prognostic index (CLL-IPI). Although the robustness of CLL-IPI has been confirmed in different validation studies it remains unclear whether CLL-IPI has the greatest validity and should be preferred to guide clinical decision in CLL.

**Aims:** To shed light on this important research question, we conducted a systematic review which includes all published studies which used CLL-IPI to prognosticate overall survival (OS) in CLL.

**Methods:** A comprehensive MEDLINE search using “CLL-IPI” as Medical Subject Headings (MESH) allowed to identify at the cut-off time of February the 28, 2017 “seven hits” with only “four” citations considered pertinent. The search was extended to the conference proceedings of annual meetings of ASH, EHA and ASCO of last two years recognized “three” additional citations.

**Results:** Overall 6720 patients from seven evaluable studies were suitable for the present analysis aimed at assessing the impact of CLL-IPI on OS. The majority of patients (4635 or 73.7%) came from studies of external validation of CLL-IPI while 17% (919) and 8.5% (576) had been used to generate (training) and to internally validate the model. Patient distribution into the four risk categories of CLL-IPI was heterogeneous thus reflecting the CLL phase (i.e., at diagnosis, at time of first treatment and at relapse) of patients within different studies. Accordingly, patients diagnosed as having low-, intermediate-, high- and very high-risk CLL-IPI ranged respectively between 9% and 58%, 25% and 39%, 14% and 52% and 2% to 9%. Next we evaluated the 5-year OS of patients stratified into each of the four CLL-IPI risk groups using either “O” or “P” test to assess the heterogeneity across different studies. The 5-year survival probability was 91% for low-risk group (95% CI, 90-91%; Q=5.2; P=0.02; I2=66%), 60% for high-risk group (95% CI, 57-62%; Q=18.1; P=0.01; I2=67%).

**Summary/Conclusions:** In this comprehensive review and meta-analysis of studies thus far published on CLL-IPI we confirmed the value of this novel model to predict OS whatever the CLL phase (fig 1). The prognostic impact of CLL-IPI extends an extensive validation in patient cohorts receiving therapy with B-cell receptor or bcl-2 inhibitors. Nonetheless, in a study of relapsed/refractory CLL included in this analysis the PI3K-inhibitor idelalisib was not able to overcome the impact of CLL-IPI risk categories on OS.

**E1022**

**IBRUTINIB FOR CHRONIC LYMPHOCYTIC LEUKEMIA: IMPACT OF THE CANADIAN YOU&I PATIENT SUPPORT PROGRAM ON TREATMENT ADHERENCE**

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**Figure 1.**

**Summary/Conclusions:** This comprehensive review and meta-analysis of studies thus far published on CLL-IPI we confirmed the value of this novel model to predict OS whatever the CLL phase (fig 1). The prognostic impact of CLL-IPI extends an extensive validation in patient cohorts receiving therapy with B-cell receptor or bcl-2 inhibitors. Nonetheless, in a study of relapsed/refractory CLL included in this analysis the PI3K-inhibitor idelalisib was not able to overcome the impact of CLL-IPI risk categories on OS.

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**haematologica | 2017; 102(s2) | 419**
Background: Oral anticancer medications (OAMs) present several advantages compared with intravenous cytotoxic chemotherapy, including greater convenience for the patient. However, OAMs require that a patient be actively involved in regular drug administration over an extended period of time (Schneider SM, et al. Semin Oncol Nurs. 2011;27(2):133-141). Adherence to OAMs significantly impacts patient outcomes; poor adherence may result in inferior survival and outcomes, higher hospitalization rates, treatment resistance, and increased healthcare costs (McDade DA, et al. Pharmaceutical. 2014;34(5):481-494).

The Canadian YOU&i™ patient support program (PSP) was developed to improve adherence to long-term ibrutinib therapy using research-proven techniques for promoting positive behavioral changes, i.e. cognitive behavioral therapy, psycho-social support, and a nurse coaching component. Results from the program are presented below.

Aims: To evaluate patient adherence to ibrutinib, and patient and physician satisfaction with the YOU&i™ PSP

Methods: Using evidence-based literature reviews and global/local market research, various patient-centered barriers to treatment adherence were identified. The YOU&i™ PSP score was calculated using the Morsky Medication Adherence Scale© score, which informed nurse coaching frequency. Adherence was delineated by prescription refill compliance. Patient and physician questionnaires were used to gauge satisfaction with the YOU&i™ PSP.

Results: As of 20 January 2016, a total of 903 patients with CLL were enrolled in the YOU&i™ PSP. A total of 552 patients were included in the adherence analysis. Of these, 86% opted in to receive the nurse coaching component. At 2 months from treatment initiation, patients who received nurse coaching demonstrated an adherence rate of 92.3%, as compared with 63.5% for patients who did not receive nurse coaching (95% CI, 17.5-41.0; p <0.0001). At 3 months the adherence rates were 89.9% vs 60.8% (95% CI, 17.5-41.4; p <0.0001). By 9 months, adherence rates were 81.7% vs 71.1% (95% CI, -4.4 to 28.4; p =0.14). At study conclusion, 12 month adherence rates were 76.6% vs 72.2% (95% CI, -18.9 to 32.4; p=0.715). Discontinuation rates were similar in all patients, regardless of nurse coaching status at 9 and 12 months. Patients reported satisfaction rates of >90% in surveys conducted at both 3 months and 12 months of program enrollment. Of physicians surveyed at 3 months, 96% reported that the YOU&i™ PSP was helpful in supporting patient needs.

Summary/Conclusions: The current analysis provides insight into adherence patterns of patients on long-term ibrutinib treatment. These results are consistent with the literature showing that PSPs like the YOU&i™ PSP can help to improve adherence rates (Schneider SM, et al. J Adv Pract Oncol. 2014;5(3):163-172). The information obtained from long-term adherence data can help to inform future trials examining patterns of adherence with OAMs. Nurse coaching may be helpful in supporting early adherence by addressing side effects that occur more frequently at treatment initiation. Moreover, changes in disease or health status that arise over the first 12 months of therapy may provide information that allows a PSP to adapt to patients' evolving needs over the treatment journey. A better understanding of long-term adherence patterns may allow programs such as the Canadian YOU&i™ PSP to target adherence support more precisely, thereby optimizing patient outcomes.

E1023

TREATMENT AND 17P DELETION TESTING PATTERNS IN COMMUNITY PRACTICE FOR PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) IN THE UNITED STATES

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Background: CLL is the most common type of leukemia in adults in the US. Traditionally, chemotherapy and chemo-immunotherapy (CIT) have been the treatment mainstay. Historically, CLL patients (pts) with high-risk genetic features (e.g., 17p deletion (del17p)) have poor prognosis and few treatment options. Over the past few years, oral-targeted therapies have been approved in the US for CLL pts, including those positive for del17p.

Aims: This study used an electronic health record database to characterize treatment and del17p testing patterns in the first 2 lines of therapy (LoT) for CLL pts initiating treatment between 2011–2016. The association between del17p testing and utilization of targeted therapies was also explored.

Methods: This was a retrospective observational study in CLL utilizing a large, longitudinal, demographically and geographically diverse database of US cancer pts (Flatiron Health 12/2016). An analytical cohort of pts treated at community practice sites who initiated 1st LoT after Jan 2011 was developed. Two sub-cohorts of pts who initiated 1st LoT before and after 2014 were also identified to reflect the approval times of oral-targeted therapies in the US.

Results: As of Dec 2016, 3,140 pts with CLL were included in the broad Flatiron Health CLL cohort. The results of this analysis are based on the analytical cohort that included 1,700 pts who initiated 1st LoT during 2011–2016, of which 1,134 (66.7%) pts initiated therapy after 2014. Second LoT was initiated in 622 pts (36.6%) before 2016, with 174 (27.9%) of those initiations occurring after 2014. The median age at CLL diagnosis was 66.9 years; 63.2% of pts were male; 70.2% were Caucasian. Over 2011–2016, the rate of genetic testing by cytogenetics or fluorescence in situ hybridization (FISH) before initiation of 1st LoT was 67.4%. The rate of del17p specific testing before 1st LoT was 59.1% (an increase from 38.9% in 2011 to 64.4% in 2016). Among those who were tested before 1st LoT, 12.5% tested positive for del17p. In the sub-cohort of pts who initiated 1st LoT after 2014, ibrutinib monotherapy replaced fludarabine, cyclophosphamide and rituximab (R) combination as the 3rd most frequent (13.0%) 1st LoT in pts with CLL, after bendamustine/rituximab (BR) combination (29.8%), and rituximab (R) monotherapy (17.7%). In contrast, ibrutinib became the most common (43.9%) 1st LoT in newly diagnosed CLL pts with del17p (followed by BR (24.5%) and R (7.1%). 36.6% of pts initiated 2nd LoT by December 2016. The three most common treatment sequences from 1st LoT to 2nd LoT were BR to R, B to cell receptor pathway inhibitor containing therapy (16.4%), CIT to CIT (14.9%), and immuno-therapy (IT) to IT (12.4%). Overall, the utilization of oral-targeted therapies has steadily increased since 2014, and multivariate analyses indicate that the presence of del17p is strongly associated (OR=8.7 and 2.8 for 1st and 2nd LoT, respectively) with this choice once these agents became available in 2014.

Summary/Conclusions: Considerable treatment pattern changes were observed for CLL pts in the US community practice due to the adoption of newly approved targeted therapies. Presence of del17p is strongly associated with choosing a targeted therapy regardless of LoT. Future research is needed to determine how differences in pt and disease characteristics and cytogenetic testing patterns influence treatment decisions and associated outcomes.
pared between ibru and RW treatment using patient-level data from RES-
ONATE-2™ (n=136) and pooled patient-level data from the two cohorts. To
adjust for differences in patient characteristics between the trial population
and both cohorts, a multivariable Cox proportional hazards model was fitted on
patient-level data to estimate the hazard ratio (HR) for ibru vs RW treatment,
with age, sex, disease stage (based on Rai/Binet), and deletion 11q pres-
ence/absence included as covariates.

Results: Median age at treatment initiation for CLEARLE (n=418) and Lyon-Sud
(n=110) was 73 and 71 years, respectively, vs 73 for ibru patients from RES-
ONATE-2™. The proportion of male patients was 63% in CLEARLE and 57% in
Lyon-Sud vs 65% in RESONATE-2™. The median follow-up was 35.7 months
(mo) for Lyon-Sud and 16.8 mo in CLEARLE vs 29.1 mo for RESONATE-2™.

Adjusted HR for ibru vs physician choice in CLEARLE and Lyon-Sud were 0.23
[95% CI: 0.14, 0.39] and 0.25 [0.14, 0.43] for PFS, and 0.29 [0.11, 0.79] and 0.39
[0.18, 0.83] for OS, respectively. Fludarabine+cyclophosphamide+ritux-
imab (FCR; n=117), bendamustine+R (BR; n=91), CHL alone (n=43), CHL+R
(n=45), and other R-containing regimens (n=154) were the most commonly
used treatment regimens across both RW cohorts. Older age, male gender,
developed disease stage and del(11q) positive status were independent risk fac-
tors for PFS and OS. The adjusted HRs (pooled estimates) for ibru vs the two
most commonly used regimens were 0.30 [0.17-0.53] (FCR) and 0.33 [0.16-
0.68] (BR) for PFS, and 0.44 [0.20-0.95] (FCR) and 0.33 [0.13-0.83] (BR) for
OS (Figure 1). Estimates of HR vs regimens in the cohorts were consistent
across both databases.

Figure 1.

Summary/Conclusions: This adjusted comparison of patient-level data from
RESONATE-2™ with RW data from CLEARLE and Lyon-Sud demonstrates ibru
to be more effective compared with RW treatment, with a 4.1-fold improvement
in PFS and a 3-fold improvement in OS. When comparing ibru with the most
commonly used RW treatments, statistically significant benefits for ibru were
consistently observed vs all treatment regimens on PFS and for most compar-
isons on OS. These results further support the existing evidence that ibru sig-
nificantly improves PFS and OS vs common regimens used in TN CLL settings,
and has important implications for clinical practice.

E1025

CHARACTERISTICS, TREATMENT, AND OUTCOMES OF ≥80 YEAR OLD PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) ENROLLED TO PROSPECTIVE TRIALS OF THE GERMAN CLL STUDY GROUP (GCLLSG)

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Background: People over 80 are the fastest growing age group in western
populations. Clinical management of ≥80 year old patients (pts) with CLL
remains a challenge due to the very limited amount of data currently available
for this age segment. Two retrospective studies reported observational data on
characteristics, treatment, and outcomes of ≥80 year old pts not enrolled in a
clinical trial (Bachmann et al. 2016). Meunier et al. 2016) is a little known about ≥80
year old pts who were treated for CLL within clinical trials, however.

Aims: To study the characteristics, treatment, and outcomes of pts aged ≥80
years who received their first therapy within prospective trials of the German
CLL Study Group (GCLLSG).

Methods: Trial populations of seven clinical trials of the GCLLSG (CLL1, CLL5,
CLL7, CLL8, CLL9, CLL10; N=3552) were reviewed and screened for ≥80 years at frontline treatment. Clinical, laboratory, and genetic data of
identified pts were pooled. Time-to-event data were analysed by Kaplan-Meier
methodology. Independent prognostic factors for survival were identified by
multivariate analysis using Cox regression modelling with stepwise selection
procedures.

Results: Among 3552 reviewed GCLLSG trial participants, 152 were aged ≥
80 years at initiation of firstline treatment. A majority of these pts were identified
from CLL1 (n=132) while the remaining were from CLL1 (n=3), CLLS (n=1),
CLL7 (n=3), CLL8 (n=2), CLL9 (n=9), and CLL10 (n=2). Median age was 82
years (range 80-90). Concomitant diseases were present in 99% of the pts
and median cumulative illness rating scale (CIRS) score was 8 (0-18). Median
creatinine clearance was 46 ml/min (range 17-99 ml/min). Identified genomic
aberrations were 13q deletion as a sole abnormality in 27%, trisomy 12 in 18%,
11q deletion in 9%, and 17p deletion in 16% of pts. (IGHV was unmutated
in 69% of the pts. Distribution of CLL-IPI risk groups was as follows: 6% low, 19%
intermediate, 61% high, and 14% very high. Most pts had Binet stage B (36%)
or C (43%). Chemomoimunotherapy with chlorambucil plus obinutuzumab (CLB-
OB) or chlorambucil plus rituximab (CLB-R) was administered to 61 (40%) and
56 (37%) pts, respectively. Remaining pts received chlorambucil alone (CLB,
n=19), fludarabine (F, n=10), fludarabine/cyclophosphamide (FC, n=1), fludara-
bine/cyclophosphamide/rituximab (FCR, n=2), or bendamustine/rituximab (BR,
n=3). Rates of grade 3 or 4 neutropenia and infections were 35% and 13%,
respectively. Premature treatment discontinuations occurred in 15% of cases
and were mostly due to adverse events. The total overall response rate was
92% with 13% complete remissions. Median observation time for all pts was
40.7 months. Median progression-free survival (PFS) and treatment-free sur-
vival (TFS) were 17.2 and 32.3 months, respectively. A total of 47 pts (31%)
received at least one further line of treatment. Median overall survival (OS)
was 48.3 months, with 22% (22%) and progressive CLL (16%) being the
most frequent causes of death. Standardized mortality ratio was calculated
and showed a 1.99 (CI 1.54-2.53) increased risk of death as compared to an
age- and sex-matched general population. Independent prognostic factors for
OS were 17p deletion and elevated serum thymidine kinase.

Summary/Conclusions: Findings suggest that antileukemic therapy (including
chemoimmunotherapy) is feasible and efficacious in ≥ 80 year old pts with CLL.
However, such pts are still highly underrepresented in clinical trials and even
with modern treatment live shorter than age-matched controls of the general
population. Broader recruitment of these pts to prospective trials and evaluation
of targeted therapies therefore appears imperative to improve outcome of CLL
in this age segment.

E1026

THE ROLE OF CD200 IN THE DIAGNOSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Clinical, morphologic, immunophenotypic and genetic features are
the basis for the diagnosis of B-cell malignancies. It is considered that the
diagnosis of CLL requires the presence in peripheral blood of >5x109/L monoclonal
B lymphocytes with a distinctive immunophenotype (i.e. SmIgmut+, CD5+, CD23−,
CD19+, CD200). Based on immunophenotypic characteristics, Matutes et al
described in 1994 a immunophenotypic score based on a few markers (CD5+
CD23−, FMC7−SmIgmut+and CD22+) each one of them receiving a score of 1
if present or 0 if absent. A total score of 4 or 5 is typical of CLL whereas those
cases scoring 0 or 1 correspond to other B-cell malignancies, mostly lympho-
plasmas. Nevertheless, clinical and immunophenotypic features of CLL may
overlap with other B-cell malignancies. CD200 has been described as a marker
shared by chronic lymphocytic leukaemia and other B-cell malignancies. CD200
therefore potentially useful to distinguish CLL from other B-cell malignancies.

Aims: The aim of this study was to analyze whether the addition of CD200 to
the Matutes score improves the diagnostic accuracy of CLL.

Methods: We prospectively assessed the immunophenotype of 99 peripheral
blood samples of patients with suspected lymphoproliferative disorders
between November of 2015 and January of 2017. Immunophenotyping was
performed using a Canto Flow Cytometer (Becton Dickinson) and samples
were stained with routine combinations plus CD200. The Matutes Score
was calculated as follows: FM7C, CD22 and CD79b were considered score 1
in CLL. The positive cells were <30%. CD5 and CD23 were considered score 1
when the positive cells were >30%. The cut-off used for CD200 was
calculated by Receiver Operating Characteristics (ROC). CD200 was scored
as 1 when the positive cells were >96%. Mean Fluorescence Intensity Ratio

Figure 1.
MICROARRAY TECHNIQUES FOR THE DETECTION OF COMPLEX KARYOTYPE IN CHRONIC LYMPHOCYTIC LEUKEMIA: A SIMPLE YET POWERFUL TEST CORRELATING WITH CLINICAL OUTCOME AND MINIMAL RESIDUAL DISEASE

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Background: An abnormal serum Free Light Chain (sFLC) ratio has been shown to be significantly associated with poor outcome in chronic lymphocytic leukemia (CLL). Yegin ZA et al, Eur J Haematol 2010, suggesting that this parameter may discriminate different biological subgroups.

Aims: As the technic is easily implementable in routine lab and cost effective, we evaluated the sFLC levels (kappa + lambda) and kappa/lambda (K/L) ratio in CLL patients in this prospective study. The relationship between abnormal sFLC levels (K+L) and K/L ratio, minimal residual disease (MRD) assessed by flow cytometry (FCM) and disease evolution was evaluated.

Methods: Diagnosis was confirmed by 10-color FCM immunophenotyping of blood lymphocytes on a Navios (Beckman Coulter). Serum FLC kappa and lambda chains were measured by nephelometry using the Freelite™ immunoassay. The normal free kappa chains level was defined as within the range of 3.3-19.4mg/L, and the normal lambda chains level within the range of 5.71-26.30mg/L. A normal sFLC kappa/lambda (K/L) ratio was therefore defined as between 0.26 and 1.65 (a ratio above 1.65 indicating an excess of kappa light chain, and a ratio below 0.26 indicating an excess of lambda light chain). The cumulative level of kappa plus lambda (K+L) was also evaluated. Most patients received combined chemo-immunotherapy or entered clinical trials whenever possible. The ROC methodology was used to establish the best cut-off value of sFLC ratio level to discriminate treated patients from those who remained treatment-free.

Results: Main patients characteristics are detailed [N=147, M/F:75/72, 111 in early disease, 36 in advanced disease, 17 in relapse]. Patients were classified into 5 groups according to the FISH scores. FISH score one was defined as 0% abnormality, score two as 1-10%, score three as 11-50%, score four as 51-90%, and score five as 91-100% abnormality.

Summary/Conclusions: The number of chromosomal abnormalities detected in CLL patients differs if assessed by FISH or micromosaic microarrays. The current 5Mb cut-off to define clinically relevant CNA should be revised, as it could underestimate genomic instability (contiguous small deletions, chromothripsis). More studies should be performed to establish standard criteria for diagnostic stratification of CLL patients based on genomic complexity consistent with the results from both techniques.
Aims: To evaluate the impact of HYPO and single Ig classes on TTFT in a retrospective cohort of CLL patients and to assess the relationship between HYPO and CLL-IPI.

Methods: We retrospectively evaluated 698 consecutive CLL patients diagnosed at our Institution from 1983 till 2016. Data from laboratory, biological analysis and clinical stage were collected. We also evaluated immunoglobulin (Ig) subclasses (IgG, IgM, IgA) at diagnosis and calculated CLL-IPI. HYPO was defined basing on our laboratory cut-offs (IgG 70mg/dl, IgG 700mg/dl, IgM 40mg/dl). However, as no recognized prognostic/predictive Ig cut-off has been reported to date, we aimed to identify a prognostic threshold for each Ig class.

Results: From 698 patients assessed, 410 cases were evaluable for Ig levels at diagnosis. IgA levels were lower than 70mg/dl in 17.4%, IgG lower than 700mg/dl in 22.2%, and IgM lower 40mg/dl in 33.7%. Forty-six percent of patients presented deficit of at least one Ig class, while 7.8% of patients had all Ig low. Each Ig deficit was related with a shorter TTFT with the following hazard ratios (HRs): 2.09 (1.45-3.03) for IgA (P<0.0001), 1.58 (1.10-2.27) for IgG (P=0.008) and 1.52 (1.09-2.13) for IgM (P=0.01) (Figure 1, A-B-C). However, only IgA deficit maintains statistical significance in multivariate analysis [HR 1.59 (1.08-2.35)]. A prognostic threshold for each Ig class was identified maximizing the differences in TTFT and the following values were obtained: 80mg/dl for IgA, 410mg/dl for IgG and 18mg/dl for IgM (Figure 1, D-E-F). Considering all 18 patients presented IPI 0, 99 had IPI 2-3, 32 patients IPI 4-6, and 12 patients had IPI 7-10. Even in our series, CLL-IPI separated four risk groups with different TTFT and OS, suggesting that our cohort may be suitable to evaluate new prognostic factors. As regards the relationship between HYPO and CLL-IPI, we observed a correlation with IgA levels, using our laboratory cut-offs. Moreover, we found a relationship among CLL-IPI and both IgA and IgM values, when using the newly validated Ig cut-off. Finally, CLL-IPI was a stronger prognostic factor for TTFT than HYPO in our analysis. However, the addition of IgA deficit to CLL-IPI appears to further improve CLL prognostication.

Figure 1.

Summary/Conclusions: In conclusion, HYPO significantly impacts on CLL prognosis. Moreover, even if CLL-IPI has a stronger prognostic value for TTFT compared to HYPO, the addition of IgA deficit appears to further improve CLL prognostication.
E1031

CLL: IS LYMPHOCYTE DOUBLING TIME (LDT) A RELEVANT PROGNOSTIC PARAMETER IN THE ERA OF PROGNOSTIC BIOMARKERS?


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Background: In CLL, tumor doubling time is reflected by the pace at which lymphocytes increase in blood (lymphocyte doubling time or LDT). However, since LDT is rarely available at the time of diagnosis, its role in assessing prognosis in patients in CLL is controversial.

Aims: To reassess the prognostic significance of LDT in a large series of patients.

Methods: Retrospective single-center study based on 629 patients diagnosed with CLL/SLL. LDT was measured at the time of diagnosis if prior WBC counts were available or calculated after diagnosis by linear regression analysis, usually over a treatment-free period of 2 months and including at least three WBC counts.

Results: 140 patients displayed short LDT (<12 months) and 489 long LDT (>12 months). The median follow-up was 13.4 years (6.1-22.5) and 11.2 years (2.3-30.9), respectively. Patients with short LDT were younger (p<0.005), had a higher percentage of clinical stage (p=0.001), higher ANC (p=0.001), as well as increased serum LDH (p=0.001) and B2-microglobulin (B2M; p=0.035) levels and also a tendency towards lower levels of Hb and platelet counts. A short LDT was also associated with an increased expression of ZAP70 and CD38, unmutated IGHV (all p<0.005) and poor FISH cytogenetics (del17p, del11q) (p<0.001). Additionally, patients with a short LDT presented more frequently mutations in NOTCH1 (p=0.008), ATM (p=0.029), TP53 (p=0.035) and a tendency to more mutations in SF3B1 (p=0.102). The proportion of patients treated in each group was markedly different [80% vs 46%] as it was the median time to treatment (TTT, 1.4 vs 9.4 years; p<0.001). Type of treatment (main, chemotherapy, immunotherapy and purine analogues) did not significantly differ between both groups and there was no significant differences in response rates (ORR 59% with 29% CR vs 69% with 29% CR; p=0.253). Overall survival (OS) was shorter in the group with short LDT (median: 7.2 vs 12.2 years; p<0.001). Univariate analysis demonstrated a significant correlation between OS and advanced clinical stage, age >70 years (p<0.001), increased B2M and LDH (p<0.001), short LDT, TP53 mutations (p=0.029), ZAP70 and CD38, unmutated IGHV, and high-risk FISH genetics (del17p, del11q) (all p<0.001). Likewise, mutations in NOTCH1 (p<0.001), SF3B1 (p=0.027), ATM (p=0.028) and TP53 (p=0.02) were associated with OS. In a multivariate analysis including clinical stage, age, LDT, IGHV, ZAP70, FISH cytogenetics and TP53, LDT (HR 1.5, 95% CI=1.3-1.6) was an independent predictor of OS (p<0.001). There was a significant correlation between OS and short LDT for patients with IGHV unmigual (p=0.001), and between OS and short LDT for patients with TP53 mutational status (p<0.001), as well as a significant decrease in OS for patients with NOTCH1 mutations (p=0.001) and patients with SF3B1 mutations (p<0.001).

Summary/Conclusions: LDT is a relevant prognostic parameter in the era of novel agents, and should be considered in CLL patients.

Figure 1.

Summary/Conclusions: All IIT are still regarded as equally important, although no solid evidence exists to support such statement. In our series, infiltrative cytopenia and/or progressive lymphadenopathy/splenomegaly constituted the IIT in most (85%) CLL patients. In spite of being enriched in favorable biological prognostic factors (mutated IGHV genes, low ZAP70 expression and favorable-risk cytogenetics), MF patients had a shorter age-adjusted OS from first-line therapy compared to LM patients. Further studies should address whether this result also applies to patients treated with novel agents.

E1032

INDICATIONS FOR TREATMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA: CLINICO-BIOLOGICAL CHARACTERISTICS AND PROGNOSTIC IMPACT

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Background: Chronic lymphocytic leukemia (CLL) is characterized by the progressive accumulation of monoclonal CD5+ B cells in the bone marrow and lymphoid tissues. International guidelines recommend initiation of treatment only in case of refractory cytopenia, progressive splenomegaly or lymphadenopathy, short lymphocyte doubling time (LDT), B symptoms and/or reduced performance status (PS) (IC). These criteria are based on experts’ consensus and considered equally relevant for treatment initiation, even though little evidence exists concerning the relative value of each individual criterion.

Aims: To describe the clinico-biological characteristics and prognosis of CLL patients according to the criteria that prompted the initiation of first-line treatment.

Methods: A retrospective study of consecutively treated patients with CLL who received first-line therapy from 1978 to 2014 and had their indication(s) for treatment (IIT) recorded. We decided to focus on these two groups. Patients whose IIT was both LM and MF were classified as MF following the logic behind Rai and Binet staging systems.

Results: Median age of the whole cohort was 62 years (range 22-93), and 63% of patients were male. Median follow-up from first-line therapy was 64 months. Type of treatment was predominantly chemotherapy, and 50% of patients were initiated therapy with an IIT of LM. MF or both, we decided to focus on these two groups. Patients whose IIT was both LM and MF were classified as MF following the logic behind Rai and Binet staging systems.

Figure 1.

Summary/Conclusions: All IIT are still regarded as equally important, although no solid evidence exists to support such statement. In our series, infiltrative cytopenia and/or progressive lymphadenopathy/splenomegaly constituted the IIT in most (85%) CLL patients. In spite of being enriched in favorable biological prognostic factors (mutated IGHV genes, low ZAP70 expression and favorable-risk cytogenetics), MF patients had a shorter age-adjusted OS from first-line therapy compared to LM patients. Further studies should address whether this result also applies to patients treated with novel agents.

E1033

UNCOVERING PRIMARY TP53-DELETED CLONES WITH FISH THROUGH FACS-SUPPORTED PURIFICATION OF CHRONIC LYMPHOCYTIC LEUKEMIA LYMPHOCYTES

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Background: The presence of TP53-inactivation in chronic lymphocytic leukemia (CLL), namely through the absence of all or part of the chromosomal region containing its locus, is a well-established marker of poor prognosis and chemoresistance to traditional chemotherapeutic agents. Fluorescence in situ hybridization (FISH) is a useful tool for the detection of the deletion. Nevertheless, its sensitivity is influenced by the number of blood-cell lineages that carry the aberration, the absolute count of deletion-positive cells, and the proportion of deletion-positive neoplastic cells relative to deletion-negative neoplastic cells and non-neoplastic cells, in the whole blood or bone marrow sample. The latter issue can be minimized by purifying the sample through the selection and separation of tumor cells, using techniques such as fluorescence-activated cell sorting (FACS).

Aims: In this study, we aim to evaluate the benefit of using purified samples of neoplastic CLL lymphocytes for the detection of TP53-deletion by FISH, when compared to full samples.
Methods: We reviewed all CLL samples that were submitted for the investigation of TP53-deletion through FISH, in our Lab, between January 1st 2011 and February 28th 2017. Results obtained on tests performed on whole mixed cellularity samples were compared with results obtained directly in FACS purified CLL clonal lymphocytes.

Results: We analyzed 40 samples tested for the deletion of TP53 in our Lab during the study period. The majority of patients (63.2%) were male. Although FACS separation of neoplastic cells was only introduced within the last two years of the study period, it accounted for 39.0% of all tested samples. This poor prognostic aberration was identified in 15.8% of patients in the overall cohort, with no differences in the incidence of a positive finding between mixed cellularity samples and FACS purified samples (15.6% vs 16.2%, respectively, p=NS). In contrast, the average proportion of positive cells within a positive sample was markedly different between mixed cellularity samples and FACS-processed samples, increasing nearly three-fold through the purification of the sample, from 24.0±15.9% to 62.9±33.3%, p<0.001. In fact, in 57.7% of all patients who were tested after FACS separation of CLL cells, the TP53-deleted clone was larger than 50% of neoplastic clonal lymphocytes, making it the primary clone.

Summary/Conclusions: We observed that the pre-processing of the sample through the FACS-supported purification of CLL neoplastic lymphocytes revealed that the TP53-deleted clone was nearly three-fold larger than suggested by the mixed cellularity sample, increasing from an average of a quarter of all cells, to nearly two-thirds. This finding uncovered that the TP53-clone was, in fact, the primary major clone within the neoplastic lymphocyte population in the majority of patients. Considering the poor prognosis conferred by the aberration, and its impact on current treatment decisions, it is quite significant to correctly identify a primary deletion-positive clone, instead of mislabeling it as a secondary minor clone.

E1034
PRIMARY PEGFILGRASTIM PROPHYLAXIS VERSUS FILGRASTIM GIVEN “ON DEMAND” FOR CLADRIBEINE-INDUCED NEUTROPENIA IN HAIRY CELL LEUKEMIA
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Background: Major advances in the treatment of patients with HCL were made in the 1980's after the introduction of two purine analogues: pentostatin and cladribine. Both these agents dramatically altered the clinical course and outcome of this disease and induced high response rates of 75-90%, with durable remissions and subsequent median relapse-free survival of up to 15 years. The major significant short-term toxicity of therapy with cladribine are neutropenia and neutrophenia (NF). Based on the script data: 71% of patients experienced grade 4 neutropenia (absolute neutrophil count [ANC] <500×10⁹/L), and 42% develop NF. The latter complications may result in life - threatening infections, as well as hospitalization.

Aims: In this retrospective study, we compared the incidence and duration of neutropenia, NF and hospitalization in patients with HCL treated with cladribine followed by pegfilgrastim as primary prophylaxis versus daily filgrastim given on demand “according to the absolute neutrophil count.

Methods: The study population included 202 patients with HCL, diagnosed and followed in 12 medical centers in Israel during 1985-2015. Patients were treated with cladribine, for 5-7 days given either sub-cutaneously or via intra- venous infusion. Medical records were evaluated for details of disease at diagnosis, including date of diagnosis, age, sex, ethnicity, complete blood count results, and spleen size at diagnosis. The efficacy of pegfilgrastim and filgrastim was assessed by evaluating the incidence of neutropenia (defined as ANC <1000×10⁹/L), number and length of hospitalizations due to NF, severity of infections and the number of days from the last day of therapy until recovery of ANC to >1000×10⁹/L.

Results: Mean follow up was 7.5 years (0.1-40), with 5 and 10 years’ survival of 96% and 90.6% respectively. The median age at diagnosis was 53 years, and 81.8% were males. First line therapy with cladribine was given to 159 patients, and of these 50.3% required hospitalization for the administration of broad-spectrum antibiotics due to NF. The risk factor to develop NF was WBC< 0.6 10⁹/l, and ANC<0.310⁹/l. Twenty eight patients were treated with pegfilgrastim as primary prophylaxis 24 hours after the last day of therapy with cladribine, while 75 patients received filgrastim on demand “due to neutropenia Median hospitalization days, and Nadir duration was 8 and 18 days respectively in both groups (p=0.71, p=0.44).

Table 1.

Summary/Conclusions: Infectious complications post cladribine treatment, remains high, with an incidence of 50.3%. For all parameters analyzed, including the percentage of febrile patients, number of febrile days, and NADIR duration the results of primary pegfilgrastim prophylaxis and filgrastim given on demand were similar. Accordingly, we conclude that it remains the treating physician’s choice to decide on which type of filgrastim to use and when to administer it.

E1035
REDUCED HEALTHCARE RESOURCE UTILIZATION IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA ACHIEVING COMPLETE REMISSION TO FIRST-LINE THERAPY
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Background: Most targeted therapies in the management of chronic lymphocytic leukemia (CLL) lead to high overall response rates but complete remissions are rare. Achieving complete remission (CR) is associated with improved clinical outcomes such as longer time to progression; however little is known about the economic benefits associated with achieving CR.

Aims: The objective of the study was to compare healthcare resource utilization among CLL patients initiated on first-line treatment who achieved CR versus those who did not.

Methods: This was a retrospective chart review study. From July to August 2016, 93 US oncologists/hematologists provided data abstracted from medical charts of their CLL patients who initiated a first-line CLL treatment between January 2010 and December 2014. The study collected patient demographics, clinical characteristics, response to first-line therapy, and the number of all-cause hospitalizations between first-line therapy initiation and end of the data follow-up (i.e., patient’s date of death, end of care, or data collection date, whichever occurred first). Patients were selected based on their best response to first-line therapy (i.e., CR, partial remission [PR], stable disease [SD] and progressive disease [PD]) as defined by the physician according to iwCLL 2008 criteria. The targeted number of patients in each category was a priori determined based on rates of response observed in clinical trials. The incidence of all-cause hospitalization was compared between patients who achieved CR and those who did not (including patients with PR, SD or PD) using univariate and multivariate generalized linear models with a Poisson distribution. As patients had different follow-up, incidence rates were reported per-patient-per-month (PPPM). Multivariate regression models were adjusted for age, gender, selected comorbid conditions, time from CLL diagnosis to first-line initiation, and Eastern Cooperative Oncology Group (ECOG) status.

Table 1.

Results: Patient-level data was collected for 179 patients who achieved CR and 151 patients who did not achieve CR (120 patients with PR, 25 with SD, and 6 with PD). Average time from CLL diagnosis to first-line initiation was 8.4 months for patients who achieved CR and 13.3 months for those who did not. The majority of patients were male (65%), the average age was 63 years, and 80% of patients had an ECOG of O or 1 at first-line therapy initiation. The medi-
an follow-up after first-line therapy initiation was 30 months. Over that period, patients who did not achieve CR had statistically significantly higher incidence of all-cause hospitalization compared to patients who achieved CR (0.021 vs 0.006 PPPM; unadjusted incidence rate ratio [IRR]=3.30, p<0.05). After adjusting for potential cofounders, the incidence of all-cause hospitalization was 2.4 times higher for patients who did not achieve CR compared to those who did (IRR=2.4, p<0.05).

Summary/Conclusions: Results from this study showed that achieving CR to first-line therapy (vs. not achieving CR) is associated with reduced frequency of all-cause hospitalizations. This suggests that, in addition to the clinical benefit associated with CR achievement, treatment strategies in CLL that improve CR may help reducing the economic burden of CLL management for both patients and payers.

E1036
RITUXIMAB (R) USED AS A SINGLE AGENT FOR AUTOIMMUNE HEMOLYTIC ANEMIA (AIHA) IN TREATMENT NAÏVE CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS INDUCES ALSO SIGNIFICANT DISEASE RESPONSE WITHOUT TOXICITY
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Background: There are very few effective treatment options for steroid refractory AIHA of CLL or for CLL patients(pts) that are unable to receive corticosteroids. R has been noted to be active in certain autoimmune hematologic disorders while experience with single-agent R in untreated CLL pts is very limited.

Aims: To report our experience concerning the use of R as a treatment of AIHA occurring during the clinical course of treatment naïve CLL pts by analyzing concomitantly its efficacy and safety as a single agent in CLL therapy

Methods: 15 pts diagnosed with CLL who received R due to AIHA were included in this study. Staging was performed at diagnosis (Binet system). Pts were placed on R at the standard dose I.V. of 375mg/m² once weekly for 6 consecutive weeks because of contraindication of corticosteroids administration.

Results: Pts’ median age was 60 (range, 42-83 y), (8 out of 15, males), 10 having disease stage A and 5 B. Two were presented with splenomegaly and 1 with B-symptoms. 12 pts (83%) had leukemic lymphocyte counts of more than 50x10⁹/L. Median time from diagnosis, the AIHA diagnosis and to 1st R infusion was 59 mos. All 15 pts completed the 6-week course of R and were assessable for response. The median WBC and the median absolute lymphocyte count(ALC) before R administration and after the end of 6-week course are shown in the Table. Resolution of the AIHA effect was achieved in all pts whereas in 4 there was a persistence of positive DAT without evidence of active hemolysis. After the 6 weekly R infusions 13 out of 15 pts (86%) showed also disease response. 12 pts experienced CR (80%) and 1 CR (6%). All pts with advanced disease also responded entering PR. Resolution of splenomegaly was documented in both splenomegaly pts. After a median follow up of 84, 5 mos from CLL diagnosis, 14 pts are alive, 9 maintain their disease response while 5 were in need of therapy due to CLL progression, after a median time of 10 mos from the last R infusion. Among them 4 were placed on FCR (2CR, 2PR) and 1 on R-Bendamustine(PR). Median PFS has not reached. All pts received the entire first dose on day 1 of treatment. There was only a grade 3 infused-related reaction in a pt with WBC>40x10⁹/L without need for hospitalization. None of the pts experienced severe tumor lysis syndrome, pulmonary insufficiency, myelosuppression or opportunistic infections.

Table 1.

Summary/Conclusions: A) R is an effective agent for AIHA treatment with concomitant significant activity against CLL and therefore could be the standard of care for CLL pts with AIHA, especially for the cohort of pts with comorbidities. B) We confirm previous data that: 1) single-agent R induces significant responses in treatment naïve CLL pts 2) R is well tolerated and its administration is not associated with myelosuppression or immunosuppression 3) R as a single agent could be an excellent first-line treatment option for pts who are very elderly or who have a poor performance status.

E1037
ATTAINMENT OF COMPLETE REMISSION IS SIGNIFICANTLY ASSOCIATED WITH IMPROVED SURVIVAL OUTCOMES IN RELAPSED/REFRACTORY (R/R) CLL: A META-ANALYSIS
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Background: Chronic lymphocytic leukemia (CLL) is an incurable neoplasm of B lymphocytes, associated with a heterogeneous clinical course. Complete response (CR) with/w/o minimal residual disease in first-line chemiomunotherapy has been associated with more favorable progression-free survival (PFS) and overall survival (OS). However, patients (pts) with R/R CLL and/or those with TP53 abnormalities (ie, 17p deletion and/or TP53 mutation) are less likely to achieve deep responses and experience poorer outcomes.

Therefore, less is known about the relationship between CR and survival outcomes in R/R CLL pts.

Aims: To quantify this association, we generated meta-analytic estimates of PFS and OS reported in clinical trials using the proportion of study patients with CR as a predictor variable.

Methods: We performed a systematic literature review of PubMed/EMBASE up to Nov 2014 and congress abstracts 2012–2014. Randomized controlled trials and observational studies evaluating any treatment in R/R CLL pts were eligible for inclusion. Data were extracted from publications as median survival, the proportions of pts surviving at specific follow-up times, or individual event and censoring times from reported Kaplan-Meier (KM) curves, along with the proportion of pts with CR. Data were synthesized to estimate overall OS and PFS including population-level CR as a covariate using a Weibull proportional hazards model within a Bayesian meta-analysis framework.

Results: 74 published studies of treatment outcomes in R/R CLL pts were identified from the peer-reviewed literature and congress abstracts. 56 of these studies reported the proportion of CRs together with either OS or PFS outcomes and were included in the analysis. Individual pt data were extracted from KM curves of 29 studies generating 5176 individual pt OS and PFS data points in addition to 54 study-level data points including 3638 pts. There were no clinically meaningful differences in study or pt characteristics among the included studies that were not also associated with CR, our variable of interest.

The hazard ratio (HR; and 95% credible interval, the Bayesian analog to confidence intervals) of survival for each 10% increase in CR among a study population was estimated to be 0.64 (0.60, 0.68). Estimated median OS for hypothesised populations with 0% CR, 25% CR, or 50% CR were 20.4 mo, 44.7 mo, and 61.9 mo. Corresponding median PFS estimates were 10.0 mo, 21.9 mo, and 30.3 mo. (Figure 1).

Figure 1. Summary/Conclusions: The attainment of CR is significantly associated with longer OS and PFS outcomes in R/R CLL at the study level. Moreover this can be expressed linearly, with each 10% increase in CR rate corresponding to a 36% reduction in the risk of progression or death. To our knowledge, this is the first meta-analysis to quantify the relationship between CR and survival outcomes in R/R CLL pts. It must be noted that these results reflect the study (population) level CR versus survival association and therefore do not necessarily represent the expected survival gain associated with an individual achieving CR. Further, CR is less likely to be achieved in pts with TP53 abnormalities, a factor not explicitly considered in our analysis. These results synthesize data from 56 clinical trials and strongly support the importance of achieving CR to improve long-term outcomes in R/R CLL pts. In particular, the prognostic association between CR and TP53 abnormalities, treatments focused on improving the likelihood of CR in these hard-to-treat pts are likely to confer the greatest impact on survival outcomes.
Aims: In this study we investigated the validity and reproducibility of these scores in a local cohort of patients with CLL.

Methods: We made a retrospective analysis including 650 unselected CLL patients newly diagnosed and previously untreated from a single institution. The final analysis has been limited to the 486 cases with complete data to apply the MDACC score, and to the 258 cases with complete data to apply the CLL-IPI score.

Results: Median age was 67 years old (25-90). With a median follow-up time of 46 months, 394 patients were alive, and 187 had received any treatment for CLL at the moment of the analysis. Median overall survival (OS) of the whole series was 173 months (127-220), and median time to first treatment (TTFT) 106 months (82-130). The MDACC score was applied to 486 cases giving 0 to 9 points to each case according to: age, b2-microglobulin levels, absolute lymphocyte count, sex, Rai stage, and number of involved lymph node groups. As shown in the Table, stratification of patients using the MDACC score allowed the prediction of prognosis for both TTFT (P<0.000) and OS (P=0.000). 162 patients were classified as low risk, 302 as intermediate risk, and 21 as high risk. Due to missing data, the CLL-IPI score could only be applied to 258 patients giving 0 to 10 points to each case according to 17p deletion, IGHV mutational status, b2-microglobulin, clinical stage, and age. As shown in the table, 126 patients were classified as low risk, 79 as intermediate risk, 46 as high risk, and 7 as very high risk. We also found significant differences in terms of OS (P=0.000) and TTFT (P=0.000) using this score.

Table 1.

Summary/Conclusions: In this study we confirm that both scoring systems are able to discriminate patients in different prognostic subgroups. Both scores are also easily applicable in clinical practice. The new CLL-IPI score is able to distinguish subgroups of patients with worse prognosis including new factors (17p deletion and mutational status of IGHV).

E1039

CHRONIC LYMPHOCYTIC LEUKEMIA: PROGNOSTIC VALUE OF CLINICAL STAGES AND CLASSICAL PROGNOSTIC PARAMETERS DEPENDING ON TREATMENT MODALITY

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Background: Prognostication is a key component in the management of patients with chronic lymphocytic leukemia (CLL). Prognostic factors however may change as a result of the introduction of more effective therapies.

Aims: To investigate whether the prognostic value of classical parameters has changed over time.

Methods: Retrospective single-center study of prognostic factors and outcome in patients with CLL diagnosed before (n=454) and after (n=903) 1995 when purine analogs and subsequently chemoimmunotherapy (CIT) were introduced in CLL treatment at the Hospital Clinic, Barcelona.

Results: The median follow-up was 8.3 years (0.1-33.0) for the overall series and 24.9 years (21.9-33.0) and 7.8 years (0.1-21.3) for patients diagnosed before and after 1995, respectively. Patients diagnosed before 1995 were older (P<0.001), had more advanced clinical stage (P<0.001), higher ARC grade (P<0.001), shorter LDT (P<0.001), and more often anemia (P=0.002), thrombocytopenia (P<0.001) and increased serum LDH levels (P=0.019) than those diagnosed thereafter. There were no differences in B2-microglobulin (B2M) levels and ZAP70 or CD38 expression. Mutated IGHV was more frequently detected in patients diagnosed before 1995 (75% vs 55%; P<0.001). The proportion of patients receiving treatment did not differ between groups (42% vs 46% (42-49%) at 6 years; P=0.08). The type of therapy given to patients diagnosed before and after 1995 was: alkylating agents (91% vs 81%), purine analogs and subsequently chemoimmunotherapy (CIT) (4% vs 4%), chemoimmunotherapy (CIT) (4% vs 5%, CIT (0% vs 31%), other (5% vs 8%) (P<0.001). The response rate was lower in patients diagnosed before 1995 (57% vs 61% (95% CI: 260; 36% GR; P<0.001) and overall survival (OS) was shorter (median 8.0 vs 10.1 years; P<0.001). The median OS in patients diagnosed before and after 1995 dropped by the following stages: stage A: 10.1 vs 10.9 years (p=0.1); stage B: 4.5 vs 9.2 years (p<0.001); stage C: 3.8 vs 8.5 years (p=0.2).

In both groups of patients univariate analyses demonstrated a correlation between OS and clinical stage (both P<0.001), age (>70 years; both P<0.001), B2M (both P<0.001), short lymphocyte doubling time (LDT) (both P<0.001), unmutated IGHV (both P<0.001), and ZAP70 (P=0.015 and P<0.001). High-risk FISH correlated with OS in patients diagnosed before 1995 (P<0.001). In patients diagnosed after 1995, the number of subjects with available FISH was too small for a meaningful analysis. In multivariate analyses (age >70 years, advanced clinical stage short LDT increased B2M, diagnosis before 1995) only age (HR 2.7 (95% CI: 2.1-3.4), P<0.001) and IGHV (HR 2.8 (95% CI: 2.2-3.8), P<0.001) showed independent prognostic significance for OS. IGHV mutational status, ZAP70 and high-risk FISH cytogenetics correlated with OS, but these variables were not included in multivariate analyses because of the many patients with missing information.

Summary/Conclusions: Survival of patients with CLL in intermediate-risk (stage B) disease has dramatically improved over the last years. In contrast, the outcome of patients with either low (stage A) or high (stage C) stage has not been significantly modified during the need for more effective therapies in these patients. Importantly, the prognostic significance of classical prognostic variables has not changed after the introduction of more effective therapies. Finally, similar studies are warranted in patients treated with novel agents.
Clinical practice in Russia. Data confirm the value of bendamustine as a first-line agent for CLL in routine therapy resulted in high rates of treatment response in the CLL. These was well tolerated in this Russian CLL population, including elderly patients.

Summary/Conclusions: First-line therapy with bendamustine plus rituximab was well tolerated in this Russian CLL population, including elderly patients and patients with renal dysfunction or other comorbidities. Additionally, combination therapy resulted in high rates of treatment response in the CLL. These data confirm the value of bendamustine as a first-line agent for CLL in routine clinical practice in Russia.

Table 1. Hematologic ADRs by CTCAE Grade.

<table>
<thead>
<tr>
<th>Hematologic ADR</th>
<th>Grade 1 (%)</th>
<th>Grade 2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia</td>
<td>49.5</td>
<td>0.0</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>21.7</td>
<td>17.5</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>2.3</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Summary/Conclusions: First-line therapy with bendamustine plus rituximab was well tolerated in this Russian CLL population, including elderly patients and patients with renal dysfunction or other comorbidities. Additionally, combination therapy resulted in high rates of treatment response in the CLL. These data confirm the value of bendamustine as a first-line agent for CLL in routine clinical practice in Russia.
Background: Diagnosis of Chronic Myeloid Leukemia (CML) implies documenting in bone marrow (BM) or in peripheral blood (PB) Philadelphia (Ph) chromosome by cytogenetics, molecular BCR-ABL fusion by FISH or BCR-ABL rearrangement by RT-PCR. In clinical practice, at the earliest, 24-72 hrs are needed to confirm CML by any of these assays. Laterly, characterization of CML stem cells (LSCs) from BM samples by FISH is needed to confirm CML or to confirm specific subtypes. Here, we investigated the accuracy and specificity of flow cytometry PB for CD26+ LSCs. Leucocytes median value was 52x10^9/L (range 5-408x10^9/L). In 83/107 (77.5%) pts we showed CD34+/CD38-/CD26+ LSCs in PB and in 83/83 (100%) the diagnosis of CML was confirmed by cytogenetics, FISH and/or BCR-ABL RT-PCR analysis. CD34+/CD38-/Lin- stem cell fraction and CD26 appeared a robust biomarker for identifying CML LSCs within the normal BM compartment. We recently demonstrated that CD34+/CD38-/CD26+ LSCs can be easily identified by flow-cytometry also in PB during treatment with tyrosine kinase inhibitors.

Aims: We investigated accuracy and specificity of flow cytometry PB for CD26+ LSCs, cytogenetics, FISH and/or BCR-ABL 1 RT-PCR analysis. CD34+/CD38-/CD26+ population was investigated in PB and when possible simultaneously in BM samples using a flow-cytometry 4-color staining procedure. 2.0x10^6/leucocytes were incubated with BD Pharmingen CD45V500 (c.2D1), CD34FITC (c.581), CD38APC (c.HIT2), CD26 (c.M-A261) and negative controls. Acquisition and analysis of at least 1.0x10^6 CD45+ cells were done by FACSCanto II with DIVA 8 software (BD, Biosciences). CD26+ cells were identified by sequential gate. CD45- and CD34- gates were performed on viable cells identified by FSC/SSC light properties and CD34+/CD38- population was gated applying a narrow gate excluding all CD38+ cells (Fig.1).

Results: PB samples from 107 pts with myeloproliferative features were evaluated for CD26+ LSCs. Leucocytes median value was 52x10^9/L (range 5-408x10^9/L). In 83/107 (77.5%) pts we showed CD34+/CD38-/CD26+ LSCs in PB and in 83/83 (100%) the diagnosis of CML was confirmed by cytogenetics, FISH and RT-PCR analysis. Median value of circulating PB CD26/L = 14 (range 0.27-698) and a positive correlation with leukocyte count (p<0.01) was found. In 53/107 (49.5%) pts analyses was performed simultaneously in BM samples. All CD26+ PB-BM matched pairs (49/53) showed superimposable results in terms of absolute number of CD26+ LSCs/L (19.18 and 18.73 respectively) while the percentage of CD26+ cells within the CD34+/CD38- fraction appeared lower in BM than in PB samples (median 28.18 and 37.33; range 0.87-77.14 and 5.99-59.97 respectively). In 24/107 (22.5%) PB samples and in 45/53 BM samples CD26+ LSCs were not detected and none of these samples was found Ph or BCR-ABL1 positive. Pts with CD26 neg PB/BM samples were subsequently diagnosed as idiopathic Myelofibrosis (12 pts), Myelodysplastic/Myeloproliferative disorders (7 pts) benign neutrophilia (5 pts). Of note, we additionally studied 4 PB+BM samples of 4 Ph+ acute lymphoblastic leukemia and all scored negative for CD26+LSCs.

Summary/Conclusions: Flow-cytometry evaluation of PB CD34+/CD38- /CD26+ LSCs is a feasible, very rapid (about 3 hrs from sample handling to results) and highly specific alternative/complementary diagnostic tool for CML. To validate these data in a larger cohort of patients we are developing a pre-titrated lyophilized antibody mixture (lyotube, BD Biosciences) to maximize sensitivity and to optimize standardization and working time, with the further aim to monitor stem cells minimal residual disease in CML patients.

E1043
LIPID PEROXIDATION AND INFLAMMATORY STATUS DURING TKI TREATMENT IN CHRONIC MYELOID LEUKEMIA PATIENTS: INTERIM ANALYSIS OF A PROSPECTIVE MULTICENTER STUDY

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Background: Evidences of increased cardiovascular (CV) events, mostly atherothrombotic, in Chronic Myeloid Leukemia (CML) patients (pts) treated with some Tyrosine Kinase inhibitors (TKIs) prompted physicians to carefully evaluate CV risk factors (CVRFs) in the choice of TKI. However, the pathogenesis behind CV events during TKIs is still largely unknown and even pts without overt CVRFs incur in CV events. We retrospectively showed that an induced “inflammatory status” during nilotinib treatment, together with genetic pro-atherothrombotic predispositions may partly explain the increased incidence of CV events. This interim analysis comprised 246 pts (78% male, median age 52 years, nilotinib treated for 6.8 months, 62% in chronic phase (CP)). Median value of IL-10 levels were significantly higher at 3 and 12 months of treatment in the nilotinib cohort at 3 and 12 months of treatment, regardless of the concomitant use of CV medications. No differences in TNFα and IL6 levels during the first 12 months of treatment were detected in the 3cohort (p<0.079). Interestingly, IL-10 levels were significantly higher at 3 and 12 months of treatment in the nilotinib and dasatinib cohort (p<0.01) respect to nilotinib (p=0.94).

Summary/Conclusions: This interim analysis, although still very preliminary, suggests that in nilotinib patients the high levels of LDL and oxLDL in combination with low levels of IL10, could induce a persistent pro-inflammatory/oxidative status potentially favoring atherothrombotic events. Additional biochemical and genetic data as well as prolonged clinical observation are needed to confirm this hypothesis. Patients enrolment and monitoring is ongoing.

E1044
TRANSCRIPTED ULTRACONSERVED NONCODING RNAs (t-UCRNS) IN CHRONIC MYELOID LEUKEMIA: EXPRESSION PROFILES ASSOCIATED WITH MOLECULAR RESPONSE TO THERAPY WITH TYROSINE KINASE INHIBITORS

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Results: We show that PAK1 and PAK2 are frequently deregulated in vivo after knockdown of individual PAKs.

Aims: In this study, we aimed at the correlation of T-UCR and miRNA-T-UCR pairs in CML, according to tyrosine kinase inhibitor (TKI) therapy, clinical risk scores and molecular response.

Methods: We analysed peripheral blood samples from 45 CML patients and 15 healthy controls. Two panels of 481 T-UCR and 752 miRNA probes were used for RT-qPCR analysis. Differential expression was evaluated using the Mann-Whitney test followed by Benjamini-Hochberg multiple testing correction.

Results: CML samples presented significantly different expression of uc.164 (p<0.01), uc.118 (p<0.01), uc.125 (p<0.01), uc.391 (p<0.01), uc.141 (p<0.01), uc.143 (p<0.05) and uc.145 (p<0.05), when compared to healthy controls. This latter T-UCR (uc.145) was associated with development and immune regulation pathways. We analysed Sokal, Hasford and EUTOS risk scores and found uc.236 (p<0.0001), uc.39 (p<0.05) and uc.7 (p<0.05) to be associated with EUTOS low risk. Concerning therapy, dasatinib was correlated with uc.236 (p<0.01), for imatinib doses, uc.4 (p<0.05) and uc.3 (p<0.05) inversely correlated with 400 and 800mg daily, respectively. Molecular response in CML samples presented a signature including uc.187 (p<0.001), uc.107 (p<0.05), uc.409 (p<0.05), uc.198 (p<0.05), uc.309 (p<0.05), uc.102 (p<0.05), uc.294 (p<0.05) and uc.361 (p<0.05). Major molecular response was identified by the altered expression of uc.198 (p<0.05), uc.215 (p<0.05) and uc.210 (p<0.05). The negative regulation of T-UCRs by miRNAs, involving T-UCR:mRNA interaction, was associated with upregulated (mir-720, mir-886-3p, mir-1274a, mir-101 and mir-129) and downregulated (mir-489 and mir-1973) microRNAs.

Summary/Conclusions: In the present study, we identified T-UCRs signatures and miRNA-T-UCR pairs associated with CML, risk scores, TKI therapy and molecular response. The expanded knowledge of RNA biology in general, together with the recent interest in the multitude of newly discovered elements such as T-UCRs, could help to improve CML therapy.

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E1046

MIRNA PROFILING OF CIRCULATING EXTRACELLULAR VESICLES IN CML PATIENTS WITH MUSCULOSKELETAL PAIN ASSOCIATED WITH DISCONTINUATION OF TYROSINE KINASE INHIBITORS

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Background: Clinical trials of TKI discontinuation are still ongoing, approximately 60% of CML patients who achieved a deep molecular response for more than 2 years maintained a major molecular response after discontinuation of imatinib. However, the long-term prognosis and/or adverse events after TKI cessation remain unclear. Recent reports showed that transient musculoskeletal pain and other muscular abnormalities occurs in approximately 30% of CML patients after stopping imatinib.

Aims: Recent evidences suggest that extracellular vesicles (EVs) that contain genetic element such as DNA, RNA, and miRNA, are important mediators of intercellular communication. We therefore studied molecular study to ascertain the possible correlation between musculoskeletal pain and EV-miRNA expression.

Methods: We investigated circulating EV-miRNAs in five CML patients who did not experience musculoskeletal events and five patients with musculoskeletal pain after stopping TKIs, as well as three healthy individuals. Peripheral blood was obtained approximately 3 months after successful TKI cessation in CML patients. Exosomes were extracted by using Total Exosome Isolation Reagent (Invitrogen, Carlsbad, CA, USA) and EV-miRNA profiling was performed with a TaqMan Low-Density Array (Thermo Fisher Scientific, Carlsbad, CA, USA), as reported previously. The relative expression level of each gene was calculated by using the comparative thresholds cycle (Ct) method. Synthetic spike control (ath-miR-159; Hokkaido System Science, Hokkaido, Japan) was used as an invariant control for EV-miRNA. This study was approved by the institutional review board of Tokyo Medical University (no. 930 approved 24 June 2008 and no. 3052 approved 9 June 2015).

Results: Three-way analysis of variance (ANOVA) performed for healthy controls and CML patients with and without musculoskeletal pain revealed EV-miR-140-3p to be the most significant value (P=0.00778). A t-test analysis using R software identified 10 differentially expressed EV-miRNAs for CML patients with and without musculoskeletal pain: seven miRNAs were upregulated (mir-107, mir-145, mir-140-3p, mir-539, mir-495, mir-299-5p, mir-425) and three were downregulated (mir-218, mir-21, mir-523) in CML patients with musculoskeletal pain. The up-regulated EV-miR-140-3p in all CML patients decreased after release of musculoskeletal pain.

Summary/Conclusions: CML patients with increased EV-miR-140-3p achieved levels similar to those of healthy controls after relief from musculoskeletal pain and inflammatory indicators in some CML patients who stopped TKIs; however, we did not find any positive association. Although the number of CML patients in this study is too small to draw definite conclusions, further research should investigate whether upregulation of EV-miR-140-3p expression in peripheral blood is correlated with musculoskeletal events in CML patients after TKI cessation.

E1047

SOLUBLE AND MEMBRANE-BOUND RECEPTOR–LIGAND IMMUNE CHECKPOINTS AND CHRONIC MYELOID LEUKEMIA: CORRELATIONS WITH MOLECULAR RESPONSE AND TYROSINE KINASE INHIBITOR THERAPY

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Background: Blockade of immune checkpoint seems to unleash the potential of the antitumor immune response in a fashion that is transforming human cancer therapeutic. Soluble and membrane-bound receptor–ligand immune checkpoints are the most druggable forms using agonist antibodies (for co-stimulatory pathways) or antagonist antibodies (for inhibitory pathways). Although its implications in immune response during chronic myeloid leukemia therapy without consistent biochemical abnormalities remains unclear, literature regarding chronic myeloid leukemia (CML) and immune checkpoint is scarce.

Aims: This study aimed at the analysis of lymphocyte subsets expression and plasmatic levels of immune checkpoint inhibitors during tyrosine kinase inhibitor (TKI) therapy in CML and its correlation with molecular responses.

Methods: Peripheral blood and cerebrospinal fluid of CML patients (n=56), divided according to molecular response to imatinib, dasatinib, nilotinib, bosutinib, ponatinib and Interferon-alpha 2b (IFN-α 2b) therapy, were included in this study. Multi-parametric flow cytometry was used for the analysis of the
expression of several immune checkpoint inhibitors (BTLA, GITR, LAG-3, PD-1, PD-L1, PD-L2, TIM-3, CD137/4-1BB) by different T, B, NK, monocyte and dendritic cell subsets. A 14-plex panel including BTLA, GITR, HVEM, IDO, LAG-3, PD-1, PD-L1, PD-L2, TIM-3, CD28, CD80, CD137 (4-1BB), CD27, and CD152 (CTLA-4) was analyzed by xMAP technology (Luminex®).

Results: Expression of CD137 by several lymphocyte subsets and PD-1 by regulatory T cells (Tregs) and natural killer (NK) cells were found significantly altered in CML patients under TKI therapy. These associations were observed for the cell population frequency expressing the receptor, and also for density of these molecules. Increased plasmatic levels of BTLA, HVEM, PD-1, PD-L1, and CD137 were associated with good molecular response to therapy. PD-1, PD-L1, TIM-3 and CD137 were found increased in patients that achieved MR4.5.

Summary/Conclusions: Some immune checkpoint inhibitors seem to be affected by TKI therapy in CML and their cell expression and plasmatic levels correlates to molecular response. Similar observations were described for other types of cancers, including solid tumors. Soluble and membrane-bound receptor–ligand immune checkpoints could represent interesting targets for future therapeutic monitoring and for pharmacologic interventions in CML.

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E1049
TYROSINE KINASE INHIBITORS SIGNIFICANTLY CHANGE THE EXPRESSION OF POLYCOMB GENES IN CHRONIC MYELOID LEUKEMIA

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Background: It has been reported that, notwithstanding their clinical success, tyrosine kinase inhibitors (TKIs) are not able to eradicate the leukemic stem cell (LSC) in patients with chronic myeloid leukemia (CML). Different mechanisms have been hypothesized, especially those linked to the niche (increased osteoblastic differentiation, angiogenesis, hypoxia...). The epigenetic control seems to be relevant, and our group previously identified a correlation between the expression of some polycomb genes (PcGs) and response to TKIs, with BMI1 resulting a good predictive molecular marker (Crea, 2015).

Aims: To better understand the role of the PcGs genes in CML patients receiving TKIs, we analyzed the expression of 86 PcGs at baseline and after 6 months of therapy.

Methods: Buffy coats obtained from peripheral blood samples of 6 patients (5 receiving imatinib and 1 dasatinib) have been used for the RNA extraction; these RNAs were used for quantifying the BCR-ABL/ABL1 ratio/αS, according to the European guidelines, and the expression of the chosen 86 PcGs by real-time PCR (PrimePCR pathway kit, Biokar, Milan, Italy) at diagnosis and after 6 months of treatment. Expression values were calculated using the 2DDCt method.

Results: At the sixth month of treatment, 5 patients were in optimal response and one was “warning”, according to the 2013 ELN guidelines. After therapy, 55% of the tested PcGs resulted up-regulated and 23% of them in the majority of patients; whereas 3 genes (DNMT3B, SCML2, CBX2) were down-regulated in at least half of samples. The expression of 5% of PcGs was “mixed”, up- or down-regulated in different samples. Among the up-regulated genes, some could be relevant from a biological point of view: 1) HLTF, a target for RUNX1, whose low expression in acute leukemia is correlated with poor outcome; 2) PHC2, able to silence the HOX genes, overcoming the multidrug resistance in myeloid models; 3) PCGF5, that is a marker of normal hematopoiesis; 4) MOV10, that has been reported to have an anti-viral activity, increasing levels of gamma interferon. This up-regulation is particularly interesting, because concerns all assessed samples and could explain our previous observation that Torque Teno virus replication does not occur in CML patients during TKIs therapy; 5) in the only “warning” patient, the up-regulation of SIRT1 was observed: this is in line with the observation that its up-regulation increases the oncogenic ability of K562 cells in a murine model. Among the down-regulated genes, could be relevant: 1) CBX2, that binding P16/p19 promotes the cell cycle progression; its down-expression could induce apoptosis; 2) DNMT3B, whose high levels have been reported in stem cells and whose reduction could characterize the differentiation process; 3) ZBTB16, whose reduction could be a sign of the reduced osteoblastogenesis, one of the mechanisms responsible for the LSC preservation in the niche; 4) SMARCA1, it too correlated to the cell cycle progression. Finally, BMI1 levels resulted unmodified in 3 cases and increased in other 3.

Summary/Conclusions: We demonstrated that PcGs de-regulation occurs in CML patients during the treatment with TKIs, with possible pathogenetic implications. Huger series of patients will improve the biological suggestions coming from these preliminary data.

E1049
IDENTIFICATION OF PROGNOSTIC AND SUSCEPTIBILITY MARKERS IN CHRONIC MYELOID LEUKEMIA USING NEXT GENERATION SEQUENCING

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Background: Chronic Myeloid Leukemia (CML) is 20% of all leukemias diagnosed every year. Discovery of Imatinib Mesylate has brought a paradigm shift in treatment of Chronic Myeloid Leukemia, despite 15% - 20% patient showing resistance to this TKI. Therefore, it is important to identify susceptibility and prognostic markers, which can help us in predicting occurrence and prognosis of CML. We did Clinical Exome Sequencing, a panel of more than 4800 clinically important genes, in CML patients

Aims: To identify prognostic and susceptibility genetic markers in CML

Methods: Enrolled CML patients (n=18) were segregated as responders (n=10) and failures (n=8) as per ELN, 2013 guidelines. Healthy controls (n=5) were also enrolled. DNA from blood of subjects was subjected Next Generation Sequencing (NGS). Mutations present in one patient group and absent in opposite group were considered as prognostic markers, whereas rare mutations, present in more than 50% of enrolled patients and absent in healthy controls, were considered as susceptibility markers

Results: We discovered mutations in genes associated with cancer or cancer related functions in different patient groups as markers. Five of them: rs116201358, rs17882014, rs4041596, rs52897880 and rs2274329 in C8A, HLA-DRB1, UNC93B1, APOH and CA6 genes respectively, were present in responders; rs4945 in MFGE8 was present in failures. Mutations in HLA-DRB1 (rs17878951, rs11554462, c.239C>G), HLA-DRB5 (rs137863146), RPHN2 (rs193179333), CYP2F1 (rs116958555), KCNJ12 (rs76684759), FUT3 (rs151218854), BM01 (rs28370522) and PRSS1 (rs144422014) were present in half or more patients

Summary/Conclusions: We discovered potential genetic markers, which can help in predicting response to IM as frontline therapy. Susceptibility markers can be used as panel for to configure individuals prone to CML

E1050
FEATURES OF THE A2455G POLYMORPHISM OF GENE CYP 1A1 IN PATIENTS WITH CML

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**Background:** Chronic myeloid leukemia (CML) is the most common myelo proliferative disorder characterized by the reciprocal translocation t(9;22) (q34;q11), leading to the formation of chimeric oncoprotein BCR-ABL on the 22q-chromosome. It is known that the protein products of the genes of cytochromes ensure homeostasis at the cellular and tissue level, carrying out the metabolism of toxic compounds that can damage the genome of the cells. Previous studies have shown that the expression of certain genes in these tumors is connected to the development of a number of neoplastic diseases, including leukemia. In individuals with weakened functional genotypes of A2425G polymorphism of CYP1A1 gene expression of this enzyme and, consequently, inactivation of xenobiotics must occur very slowly, thus creating conditions for adverse action of harmful metabolites in the genome of the cells. Currently, the scientific literature discusses the role of the negative allele A2455G polymorphism of CYP1A1 gene in the development of hematological malignancies. However, the adverse roles of genotypic variants for this gene in oncogenesis of BCR-ABL-positive patients with CML have studied not enough.

**Aims:** Evaluation the role of A2425G polymorphism of CYP1A1 gene in the formation the mutant clone of tumor and development of CML.

**Methods:** The work is performed on DNA samples isolated from the peripheral blood of the patients in the clinic of scientific research Institute of Hematology and blood transfusion in Uzbekistan. We studied 146 patients with CML. The control group was formed from 217 individuals of Uzbek nationality, without any cancer disease. The diagnosis of CML verified in accordance with the International nomenclature ISCN. Standardized PCR with detection in real-time was carried out on a thermal cycler Rotor-Gene 6000 (Corbett Research, Australia), using a set of reagents "Ambion" Leucosis quantam (M; 22) "FRT" (InterLabSers, Russia). Testing A2425 polymorphism of CYP1A1 gene was performed on a programmable thermal cycler of the company "Applied Biosystems" (USA) using test systems company "Life" (Russia) according to the manufacturer’s instructions. Statistical analysis of results was carried out using the statistical software package "OpenEpi", Version 2.3.

**Results:** The frequencies of allele A and G are as follows: 87.7% and 12.3% in patients with CML, and 93.3% and 6.7% in the control group, respectively. The frequency distribution of genotypes A/A, A/G and G/G were as follows: 76.7%, 21.9% and 1.4% - in CML patients, and 86.6%, 13.4% and 0.0% - in the control group. Observed frequencies of genotypes in the studied groups was consistent with the theoretically expected and were in equilibrium with Hardy-Weinberg equilibrium (P>0.05). There was a statistically significant decrease in carriage of the adverse alleles in the population sample comparison group patients (1.4% vs 6.7%, respectively; χ=6.8, P=0.01; OR=2.0; 95% CI 1.17-3.282). Also detected significant association of heterozygous genotype A/G in patients with CML, compared with the control group (21.9% vs 13.4%, respectively). The risk of mutant formation of the tumor clone in carriers of this genotype was 1.8 times significantly higher compared with patients not having it (χ=4.6; P=0.03; OR=1.8; 95% CI 1.046-3.166). The homozygous genotype was 1.8 times significantly higher compared with patients not having this allele (χ=6.0; P=0.01; OR=1.8; 95% CI 1.046-3.166). The homozygous genotype A/A was found with high frequency in a population-based sample of 86.6% cases of patients. At the same time, the differences reached the threshold level of significance (χ=6.8; P=0.01; OR=2.0; 95% CI 1.17-3.282). Also detected significant association of heterozygous genotype A/G in patients with CML, compared with the control group (21.9% vs 13.4%, respectively).

**Conclusions:** Our results suggest that the G allele and the heterozygous genotype A/G A2425G polymorphism of CYP 1A1 gene are important markers of increased risk in formation of malignant tumor cells and development of CML in Uzbekistan (Р<0.05). In this case, homozygous genotype A/A of A2425G polymorphism of CYP 1A1 gene has a protective character in relation to risk of CML.

**Figure 1. Evolution of hematologic toxicity grade 3-4 with time (all treatments sequences included).**

**Results:** Demographics, risk and treatment distribution: 893 patients (533 men, 360 women) with a median age at diagnosis of 52 y (14-94y) were included with a follow up of 85±7 months (m) from diagnosis, 78±6.6 m from first treatment, and 69±6 m from first TKIs. 151 patients (16,9%) were over 70y. The risk distributions were as follows: Sokal: low (L) 48%, intermediate (I) 37% and high (H) 14%; Euro score: L 50%, I 45% and H 5%; EUTOS L: 92% and H 8%; EUTOS LT: L 70%, I 23% and H 7%. Treatment groups were the following:

- **Group 1:** IFN alpha and then imatinib or 2º G TKIs (221 patients); Group 2: imatinib only (404 patients); Group 3: imatinib and then nilotinib, dasatinib or both due to failure or intolerance (177 patients) and Group 4: 2º GTKIs in first line (93 patients).

Hematologic toxicity grade III–IV. Figure 1 shows the incidence through the years (all group of treatments). From 800 patients treated with imatinib (first o second line) 67 (8.3%) had grade III–IV toxicity, and 26 had to switch treatment due to toxicity. From 166 patients treated with dasatinib (29

**Chronic myeloid leukemia - Clinical**

**E1051**

**HEMATOLOGIC TOXICITY GRADE III-IV IS ASSOCIATED WITH LOWER SURVIVAL IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH TYROSINE KINASE INHIBITORS**


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**Background:** TKIs introduction in the treatment of chronic myeloid leukemia (CML) has offered an outstanding improvement in survival outcomes. These results were obtained from clinical trials but little is known about long-term toxicity and their translation to real life. In addition, clinical trials results are mainly based on the analysis of the therapy of interest (experimental or control), but the descriptions of the subsequent treatment sequences due to failure or intolerance are normally lacking.

**Aims:** To analyze the long-term toxicity of patients outside clinical trials in clinical trials. The setting was a multicentric, hospital-based registry.

**Methods:** Toxicity grade III–IV and survival and their potentially associated variables were studied.
Poitiers, France, 4Winship Cancer Institute at Emory University, Atlanta, United
were similar in patients with and without dose reductions in each arm (table). 95
Patients on DAS maintained higher molecular response rates than
Results:
Table 1.
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by 10 years is roughly 80%, and extend the findings of our previous work show-
variate analysis (Cox model), only hematology toxicity grade III-IV and age
over 70y were independent variables.
Summary/Conclusions: 1These results show that the probability of survival
by 10 years is roughly 80%, and extend the findings of our previous work show-
ing that this probability is not different across different sequential treatments
(imatinib 1st line or post-IFN, or switched to 2GTKis due to intolerance or failure) (1).
This fact also emphasizes the rescue potential of available TKI therapies. 2.
Hematologic toxicity grade III-IV in the first two years identified a group of
patients with worse survival outcome. 3. Patients over 70 years have shorter
survival due to reasons different than progression. 4 Second GTKis showed
better hematologic toxicity profile.

Reference

E1052
5-YEAR EFFICACY OF DASATINIB AND IMATINIB IN NEWLY DIAGNOSED
PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE
(CML-CP) WITH DOSE MODIFICATIONS FROM DASISION
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Squibb, Princeton, United States

Background: Multiple dosage strengths are approved for dasatinib (DAS), per-
mitting dose-optimization strategies for patients who experience adverse events
(AEs). In a 2-year retrospective analysis of DASISION, efficacy was maintained in
DAS- and imatinib (IM)-treated patients with dose reductions or interruptions
of any cause. Aims: To evaluate the effect of dose reduction for any AE and for pleural effu-
sion on efficacy in DAS- or IM-treated patients from DASISION.
Methods: Treatment-naive patients with CML-CP in DASISION (NCT00481247) were randomized to receive either DAS (100mg once/day; N=259) or IM (400mg once/day; N=260). Dose reductions for AEs (up to 2) were allowed: DAS: 80mg, then 50mg; IM: 300mg, then 200mg. Five-year molecular and cytogenetic response rates in all patients were assessed retro-
spectively.

Table 1.

Results: Patients on DAS maintained higher molecular response rates than patients on IM, whether or not they had dose reductions for an AE; these rates were similar in patients with and without dose reductions in each arm (table). 95 (37%) DAS- and 44 (17%) IM-treated patients had dose reductions at any time
due to AEs. Median time to first DAS dose reduction was 289 days (range: 22-
2123), and median time to first IM dose reduction was 160 days (range: 31-
2052). For patients with reductions due to any cause, median average daily
dose was DAS 83mg and IM 328mg; for DAS patients with reductions due to
pleural effusion, median average daily dose was 82mg. Median duration of treat-
ment (excluding interruptions) was 54 months (range: 3-70) for patients who had a DAS dose reduction and 57 months (range: 2-71) for patients who had an IM dose reduction. Changes in level of response were tracked for patients who achieved complete cytogenetic response (CCyR) or major molecular response (MMR) before or after the first dose reduction (table). Many patients maintained or increased to CCyR or MMR following dose reductions for any AE. Hematological toxicity (9%) was the most common AE resulting in dose reduction for IM, and pleural effusion (12%) was the most common for DAS.

Summary/Conclusions: Reducing DAS doses to 80mg or 50mg was a safe and
effective means of managing patients who experienced AEs in this 5-year
retrospective analysis of DASISION. These results were consistent with previ-
ous reports and continued to show that efficacy was not affected by dose reduc-
tions for any cause, including pleural effusion. Notably, there was no loss of
CCyR following dasatinib dose reductions. Molecular responses remained higher
for DAS vs IM irrespective of dose reductions due to AEs.

E1053
EFFECT OF PLASMA TROUGH CONCENTRATION OF NILOTINIB AND
POLYMORPHISMS OF DRUG TRANSPORTER GENES ON THE FREQUENCY
OF ADVERSE EVENTS IN CHRONIC PHASE OF CHRONIC MYELOID
LEUKEMIA: STAT1 AND STAT2 TRIALS
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Background: STAT trials (STAT1 and STAT2) are multicenter, phase II, sin-
gle-treatment arm, open-label clinical studies designed to evaluate the efficacy
and safety of two-year consolidation by nilotinib (NIL) for achieving a deep
molecular response (DMR) or successful treatment-free remission (TFR) in
patients with chronic phase chronic myeloid leukemia (CML).
Aims: In this report, we focus on the adverse events (AEs), especially anemia
and liver dysfunction observed in the STAT trials. Additionally, we analyzed the
relationship between laboratory abnormalities and pharmacokinetics (PK) phar-
macogenetics (PGx) of NIL.
Methods: AEs were assessed according to the Common Terminology Criteria
for Adverse Events (CTCAE) v4.03. Safety evaluations were conducted throughout the study. Plasma trough concentrations of NIL were determined with high-performance liquid chromatography (HPLC) at 1 month (1M), 3M, 6M, 12M, and 24M in the STAT trials. Genotyping of CYP3A5*3, UGT1A1*6
(rs776746), ABCB1 [3435T>C (rs1045642), ABCB2 421C>A (rs2231142),
and UGT1A1*6, *27, and *28 was performed using polymerase chain reaction-
restriction fragment length polymorphism (PCR-RFLP). All genotype frequencies
were tested for Hardy-Weinberg equilibrium.

Figure 1.

Results: Between July 2011 and December 2012, CML patients were recruited in the
STAT trials. NIL was administered twice daily (600mg/day) for 2 years according to the study protocol. A total of 76 and 96 patients were analyzed as a safety data set in STAT1 and STAT2, respectively. In STAT1, 18 patients who achieved a confirmed DMR were switched from STAT1 to STAT2. These
participants entered both trials, but safety data had not been collected in STAT1
achieved complete cytogenetic response (CCyR) following dasatinib dose reductions. Molecular responses remained higher
for DAS vs IM irrespective of dose reductions due to AEs.

References:
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with high trough concentration of NIL (Figure 1). There were statistically significant correlations between median concentrations of NIL and the grades of each AE. Based on the results of the analysis using Cox proportional-hazards model, the trough concentration of NIL [hazard ratio=1.001 (1.000-1.002), P=0.004] and ABCG2 421A/ A [hazard ratio=3.044 (1.155-8.027), P=0.024] were independent factors for the elevated ALT. Similarly, the trough concentration of NIL [hazard ratio=1.001 (1.000-1.002), P=0.001] and UGT1A1 1/1 [hazard ratio=0.475 (0.246-0.919), P=0.027] were independent factors for the elevated total bilirubin.

Summary/Conclusions: In this study, we identified the relationship between NIL trough concentration and liver dysfunction. Our finding suggests that therapeutic drug monitoring might help avoid drug interruption and discontinuation because of AEs, especially liver dysfunction.

### E1054

**VERY EARLY MOLECULAR RESPONSE (VEMR) WITH FRONTLINE DASATINIB TREATMENT IS A STRONG PREDICTOR OF LONG-TERM BCR-ABL1 TRANSCRIPT LEVELS IN CHRONIC MYELOID LEUKEMIA PATIENTS: PCR-DEPTH STUDY**


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Background: In BCR-ABL1 tyrosine kinase inhibitor (TKI) treated chronic phase chronic myeloid leukemia (CP-CML), early molecular response (EMR) at 3 months is currently identified as being one of the most important prognostic factors. Sokal risk score and dose intensity during first 3 months were strongly associated with achievement of EMR. Although TKI is a novel drug, the kinase inhibitor with improved potency, identification of very early molecular response (VEMR) would be beneficial.

Aims: We evaluated the possibility of the VEMR at 1 month predicting long-term outcomes in newly diagnosed CP-CML patients treated with dasatinib.

Methods: In this prospective, observational, open-label study, 102 patients with CP-CML were enrolled to receive dasatinib at a dose of 100mg once daily. 44 patients with CP-CML were enrolled to receive dasatinib at a dose of 100mg once daily.

Results: Median age was 49 years (19-81 years) and 61 patients were male. With median follow-up duration of 28 months (0.9-33.8 months), 80 (78.4%) out of 102 patients were still on dasatinib treatment and 22 patients discontinued dasatinib treatment. Treatment discontinuation (n=2), progression (n=9) or adverse events (n=8) or other reasons (n=9). The BCR-ABL1 mutations, assessed in 10 patients after dasatinib discontinuation, were detected in 3 patients which were all T315I mutation. The cumulative CMR by 18 months and MMR by 24 months were 20.5% and 79.6% respectively. In safety analyses, grade 3/4 thrombocytopenia (30.3%) was most common. Pleural effusion occurred in sixteen (15.6%) patients which were mostly grade 1/2. The cut-off value of BCR-ABL1 transcript on Day+28 was calculated to predict EMR and MMR at specific timepoints.

Summary/Conclusions: Estimated OS in patients with CP-CML receiving 3L treatment prior to ponatinib appears to be shorter than that observed among ponatinib-treated patients in PACE: 4-year survival probability in PACE was higher than estimated 2-year survival probability prior to ponatinib. Further analyses are needed to identify and adjust for potentially confounding factors.

### E1056

**DETECTION AND MONITORING OF BCR-ABL1 KINASE DOMAIN MUTATIONS IN CML AND ALL PATIENTS BY NEXT GENERATION SEQUENCING AND DROPLET DIGITAL PCR, A BELGIAN PROSPECTIVE STUDY**

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Background: Among myeloproliferative diseases, development of chronic myeloid leukaemia (CML) is associated with the emergence of the fusion oncogene BCR-ABL1 resulting from a t(9,22) chromosomal translocation (Philadelphia chromosome). This chimeric transcript is also present in all acute lymphoblastic (Phi+) and Philadelphia chromosome positive (Ph+) acute lymphoblastic leukaemia (ALL) patients receiving first or second generation tyrosine kinase inhibitor (TKI) treatment. So far, the gold standard procedure to detect BCR-ABL1 kinase domain (KD) mutations is the conventional Sanger Sequencing, endowed with an analytical sensitivity of 15-20%. Recent studies on the implementation of Next Generation Sequencing (NGS) for detection of BCR-ABL1 KD mutations showed a significant dropping down of the sensitivity level (1-5%), improving patient’s treatment management.
Aims: Both NGS and droplet digital PCR (ddPCR) were used in this prospective study. NGS screened all known mutations in the BCR-ABL1 KD and ddPCR targeted only the 3 most common mutations, T315I, E255K and Y253H, which represent approximately 75% of the ABL1 mutations. Patients eligible for the study were i) CML patients with failure or warning to all lines of TKI therapy according to the 2013 ELN-guidelines, with no suspected lack of adherence and ii) ALL patients with diagnosis and/or molecular relapse. Monitoring was performed when clinically appropriate.

Methods: Total BCR-ABL1 RNA was transcribed into a long range cDNA covering the kinase and the regulatory and the SH2/SH3 domains of either p190 or p210 BCR-ABL1 transcripts (exons 4 to 10). For NGS, primers designed with the Ampliseq™ Designer Software were generated with a set of 10 ampicons. Bar-coded libraries, constructed according to the Ampliseq™ protocol, were sequenced on the Ion Torrent PGM platform (sensitivity of 2.5%). For ddPCR, cDNA was analysed for the presence of one of the 3 main mutations (T315I, E255K and Y253H). The overall number of BCR-ABL1 mutated samples was 18 (15 CML and 3 Ph+ ALL), representing 30% of the cases. Among these samples, 27 mutations were found, including 9 samples with one mutation: T315I (2), E255K (3), G250V (1), F359I (1), M237T (1) and E255A (1) and 9 harboured compound mutations: T315I + E255K (6) and T315I + Y253H (3). A high frequency (85%) of T315I, E255K and Y253H mutations was also observed (23/27). As far as these 3 mutations are concerned, reproducibility to determine mutational burden was found to be very high between NGS and ddPCR.

Summary/Conclusions: Advances in sequencing technologies and further lowering sensitivity levels contribute to optimal management of CML and Ph+ ALL patients and improve treatment outcome. The earlier a mutation in the kinase domain is detected, the earlier an informed choice can be made regarding optimal subsequent TKI treatment.

E1057

CLINICAL AND IMMUNOLOGICAL EFFECTS OF NILOTINIB IN COMBINATION WITH PEGYLATED INTERFERON-A2B IN PATIENTS WITH SUBOPTIMAL MOLECULAR RESPONSE ON IMATINIB (NORDDUTCHCML009)


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Background: The ability to ‘cure’ a proportion of chronic myeloid leukemia patients (CML) makes treatment free remission (TRF) an important treatment goal which requires sustained deep molecular response on TKI therapy. Unfortunately, not all patients achieve deep molecular responses on their first line treatment. Novel treatment strategies to increase the proportion of CML patients eligible for a TKI stop attempt are therefore needed.

Aims: The primary objective of the NordDutchCML009 study was to assess if, in CML patients with an MR4.0 after a switch to nilotinib with pegylated interferon-α2b (PegIFN-α2b) combination treatment for at least 12 months, we observed an increased number of stimulated CD3+ cells and a reduction in the proportion of CD56+ NK-cells after the addition of PegIFN-α2b to the nilotinib treatment (P<.046), but no significant changes in the other immunological parameters. In the subgroup analysis need to be interpreted with caution due to low statistical power.

Summary/Conclusions: Despite relatively poor tolerability of the scheduled Nilo/Peg combination treatment in the current study, CD56+ NK cells were significantly modulated and more than half of the patients achieved a sustained MR4.0, which would allow for a TKI stop attempt. The discontinuation rate suggests that the PegIFN dose was too high in combination with nilotinib treatment in our study population.

E1058

ANALYSIS OF VASCULAR ADVERSE EVENTS IN TKI TREATED JAPANESE CML PATIENTS: RETROSPECTIVE LARGE COHORT STUDY OF CML COOPERATIVE STUDY GROUP

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Background: Chronic myeloid leukemia (CML) is a disease of hematopoietic stem cells resulting from oncogenic chromosome translocation that leads to the formation of the BCR-ABL1 fusion gene. Treatment of chronic phase (CP) CML has dramatically changed since the emergence of the first-in-class tyrosine kinase inhibitor (TKI) imatinib, and treatment based on TKI has improved the outcome in the majority of CP-CML patients. Nowadays, second generation TKIs including dasatinib and nilotinib are continuously used, and lower disease progression rate than imatinib. On the other hand, longer treatment duration and the increased types of TKIs gave rise to various kinds of unexpected adverse events (AEs). In 2011, drug-induced peripheral arterial occlusive disease (PAOD) was first reported, followed by vascular AEs (VAEs) including ischemic heart disease (IHD) and cerebral AEs (CAEs) and peripheral occlusive disease (PAOD). Furthermore, it became clear that the incidence of VAEs increased with the dose and treatment duration, therefore VAEs are considered a more fatal complication of TKI treatment. However, there is no available data about the incidence of VAEs in Japanese patients.

Aims: We investigated the vascular safety issue and estimated the 1000 person-years risk of developing VAEs during TKI treatment, including imatinib, nilotinib, and dasatinib, using 3 risk assessment tools among 320 Japanese patients who were enrolled in the CML Cooperative Study Group.

Methods: A surveillance data of 320 patients enrolled in the CML Cooperative Study Group was conducted. Briefly, the study included patients who were diagnosed with CML-CP from April 2001 to January 2016, whose median age was 57 years old (15-80) and median time of follow up was 64.2 months. Patients in the accelerated or blastic phase (AP/BL) were excluded. The study was approved by the research ethics boards of each institutions and was conducted in accordance with the Declaration of Helsinki. All patients who developed VAEs were analyzed using 3 risk assessment tools (SCORE chart, Framingham risk score, Suita-score) to estimate the patients’ 10-year risk of VAEs.

Results: Among the 320 newly diagnosed CML-CP patients, 16 (5.0%) cases of VAEs were reported during the study period. Seven cases were treated by imatinib, 4 cases by dasatinib, 4 cases were a switch from imatinib to nilotinib, and 1 case was a switch from dasatinib to nilotinib. The VAEs were 9 IHD, 5 CI, and 2 PAOD cases. Results from the 3 risk assessment tools are as follows: SCORE (2 low, 9 moderate, 1 high, 4 very high risk), Framingham score (3 low, 5 moderate, 7 high risk), and Suita-score (13 low, 1 intermediate, 11 high risk). The 10-year cumulative rate of IHD was 4.45%, 5.26% and 2.92% in the enrolled CML-CP patients, 4.65% and 2.33% in the imatinib-treated patients, 14.34% and 10.75% in the nilotinib-treated patients, 4.08 and no case in the dasatinib-treated patients, and 1.787 and 3.342 in the age-
matched general population, respectively. Among the 320 newly diagnosed CML-CP patients, 16 (6.0%) cases of VAEs were reported during the study period. Seven cases were treated by imatinib, 3 cases by nilotinib, 1 case by dasatinib, 4 cases were a switch from imatinib to nilotinib, and 1 case was a switch from dasatinib to nilotinib. The VAEs were 9 IHD, 5 CI, and 2 PAOD cases. Results from the 3 risk assessment tools are as follows: SCORE (2 low, 9 moderate, 1 high, 4 very high risk). Framingham score (3 low, 5 moderate, 7 high risk), and Suiita-score (13 low, 1 intermediate, 1 high risk). The incidence rate of IHD and CI per 1000 person-years were 5.26 and 2.92 in the enrolled CML-CP patients, 4.65 and 2.33 in the imatinib-treated patients, 14.34 and 10.75 in the nilotinib-treated patients, 4.08 and no case in the dasatinib-treated patients, and 1.787 and 3.342 in the age-matched general population, respectively.

Summary/Conclusions: The incidence rate of IHD per 1000 person-years were higher in the nilotinib- and lower in imatinib- and dasatinib-treated CML patients, and the patients showed almost the same rate of CI as compared with the age-matched general population, even though the incidence of VAEs were lower in Japanese compared to the European cohort. More patients were estimated to have very-high and high risk of VAEs in the SCORE and Framingham score assessment tools as compared with the Suiita-score tool.

E1059
UPDATE OF CMRegistry: AN OBSERVATIONAL, MULTI CENTER, PROSPECTIVE FOLLOW-UP REGISTRY OF PATIENTS WITH CHRONIC PHASE CHRONIC MYELOID LEUKEMIA WITH A HIGH PROBABILITY OF OBTAINING A DEEP MOLECULAR RESPONSE >CMR4 (IS)
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Background: Since the introduction of Tyrosine Kinase Inhibitors (TKI), many patients diagnosed of Chronic Myeloid Leukemia (CML) in chronic phase achieve a deep molecular response. Around 50% of these patients are expected to maintain their response even after discontinuation of their TKI treatment. Several clinical trials are exploring the best way of stopping TKI therapy and evaluating patient and disease characteristics that could predict relapse after treatment discontinuation.

Aims: This is an update of the CMRegistry study aimed at collecting clinical data and molecular information from Spanish CML patients that have achieved a series of molecular milestones to any of the tyrosine kinase inhibitors who are likely to achieve, or have already achieved, a deep molecular response (>MR4) are included. This likelihood of achieving >MR4 is defined, for the purposes of the study, as a bcr/abl ratio of: 1) ≤1% at 3 months from start of TKI therapy; 2) ≤0.1% at 6 months from start of TKI therapy; or 3) ≤0.01% any time point during treatment. Clinical data have been collected using a specific CRF. All data were registered in an anonymous manner. The BCR-ABL ratios in the IS have been provided by standardized labs in Spain.

Results: From June 2014 to February 2017, 976 patients were registered in the study. Median age was 51 years (15-88). The Sokal risk groups were as follows: low risk: 345 patients (15%); intermediate risk: 307 (17%); and high risk: 129 (14%). Cytos classification yielded 714 patients in the low risk and 79 in the high risk categories. The majority of patients received first-line treatment with imatinib (626 patients), dasatinib (39 patients) or nilotinib (87 patients). Of note, 5 patients received bosutinib, 1 patient ponatinib and 74 patients were treated with Interferon previous to TKI administration. So far 14 patients have died of non-CML related conditions such as carcinoma (2 patients), ischemic heart disease, respiratory failure and sepsis. Interestingly, 2 patients developed progression of their CML to accelerated phase and blast crisis (1 patient each) with no deaths. At present, 104 patients (11%) have achieved a MR4.0, 174 patients (18%) a MR4.5 and 123 patients (13%) have obtained a complete molecular remission (undetectable bcr-abl transcripts with a sensitivity of at least 10-5).

Summary/Conclusions: Almost one thousand CML patients have been included in this Spanish prospective study owing to their promising molecular response that would predict for a sustained deep molecular remission. Four hundred and one patients have already achieved a deep molecular response (>MR4 (IS)) and could be enrolled in prospective discontinuation studies.

E1060
ANALYSIS OF DASATINIB AND IMATINIB 5-YEAR EFFICACY AND SAFETY BASED ON BASELINE COMORBIDITY AND AGE IN PATIENTS WITH NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP) IN DASISION
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Background: Patients with CML often have comorbidities, which may influence treatment-related decisions and impact response and survival. In a retrospective analysis of 1-year data from the phase 3 DASISION study, the overall safety or response in dasatinib- or imatinib-treated patients was not substantially impacted by baseline comorbidities, although certain adverse events (AEs) trended higher in patients with ≥1 vs 0 comorbidities (Khoury ASH 2010). Further analysis is warranted to determine how comorbidities may impact long-term outcomes.

Aims: To evaluate the impact of baseline comorbidities and patient age on 5-year safety and efficacy in dasatinib- or imatinib-treated patients from DASISION.

Methods: In DASISION (NCT00481247), patients were randomized to receive dasatinib 100mg/day (N=259) or imatinib 400mg/day (N=260). For this retrospective analysis, patients were grouped as having 0 or ≥1 baseline comorbidity; by baseline disorder (diabetes mellitus, hepatobiliary disease, hyperlipidemia, cardiovascular disorder, or pulmonary condition), or by age group (<46 years, 46–65 years, >65 years). Safety (treatment-related AEs in ≥10% of patients) and efficacy (response rates by 5 years and median times to response) were assessed for each group and treatment.

Table 1.
Results: The number of patients with 0 or ≥1 comorbidity was similar in the dasatinib (66 [25%]; 193 [75%]) and imatinib (67 [26%]; 193 [74%]) arms, respectively; most (>90%) patients were <65 years old. In patients with 0 or ≥1 baseline comorbidity, the median average daily dose was comparable within arms and discontinuation rates (36%-39%) were similar within and across arms (table). The overall safety profiles were comparable in the 0 and ≥1 comorbidity groups in both arms, other than specific AEs, which had a 22 times higher frequency in patients with ≥1 vs 0 comorbidities; the majority of these were grade 1/2 AEs (table). The incidence of peripheral edema increased with patient age for both imatinib and dasatinib (<46 years: 5% each; 46-65 years: 12% and 10%; ≥65 years: 21% and 20%). In this analysis, the increased incidence of pleural effusion (PE) in dasatinib-treated patients was most highly associated with increased age: <46 years (16%) vs 46-65 years (37%) vs ≥65 years (60%). PE incidence did not appear to be related to baseline pulmonary comorbidity and was similar in dasatinib-treated smokers (33%) vs nonsmokers (27%). Within each arm, patients with 0 or ≥1 comorbidity (table) and across age groups had similar responses. Discontinuation rates were higher for patients with ≥1 vs 0 comorbidities in both arms (MR4.5: dasatinib 46% vs 32%; MR4,5+imatinib 36% vs 22%). Median time to response (months) for patients with 0 or ≥1 comorbidity did not differ within each arm, but was numerically shorter for dasatinib (36 vs 35) vs imatinib (MR4.5: 42 vs 47).

Summary/Conclusions: The superior efficacy of dasatinib over imatinib was shown in previous studies. Response rates and times to response were comparable in patients with 0 or ≥1 comorbidity and trended in favor of dasatinib vs imatinib. Although a few AEs (most grade 1/2) appeared to occur at a higher frequency in patients with ≥1 vs 0 comorbidities in either treatment arm, the overall number of AEs and discontinuation rates at 5 years in patients who were treated with first-line dasatinib or imatinib did not appear to be substantially affected by baseline comorbidities.

E1062
RADOTINIB TREATMENT IN CHRONIC PHASE CHRONIC MYELOGENOUS LEUKEMIA PATIENTS WITH RESISTANCE OR INTOLERANCE TO BCR-ABL TKIS: 36 MONTHS UPDATE OF RADOTINIB PHASE 2 STUDY OF PATIENTS IN KOREA

Background: Radotinib is an orally active, selective BCR-ABL1 tyrosine kinase inhibitor (TKI), approved for the first-line and second-line treatment of chronic phase chronic myeloid leukemia (CP-CML) patients in Korea. Earlier 12 and 24 month results demonstrated that radotinib is effective and well tolerated in CP-CML patients with resistance and/or intolerance to BCR-ABL1 TKIs.

Aims: We update the long-term outcome of radotinib treatment in patients failed to BCR-ABL TKIs with a minimum follow-up of 36 months.

Methods: Ph+ CP-CML patients who failed prior TKI therapy were enrolled between July 2009 and November 2011. All patients were treated with radotinib 400mg twice daily. Cytogenetic and molecular assays were performed at base-line, every 3 months, and at treatment failure. Safety parameters were also analyzed. Probabilities of overall survival (OS) and progression free survival (PFS) were calculated using Kaplan-Meier method.

Results: A total of 77 CP-CML patients (18 years of age or over) were enrolled. This analysis includes data from last enrolled patient who received at least 36 months of radotinib therapy. With a median follow-up of 45.7 (range 0.9-65.7) months, 31 patients (40.3%) completed 36 months treatment, and 46 patients (59.7%) discontinued the treatment before 36 months. Main reasons of discontinuation were abnormal laboratory test (n=18), adverse events (n=4), treatment failure including disease progression and lack of response (n=18), death (n=2), and other reasons (n=4). Median duration of radotinib exposure was 19.5 (0.3-60.9) months. Cumulative incidence of complete cytogenetic response (CCyR) by 36 months was 90.0% and of patients achieving CCyR, 45.0% (18/40) achieved MMR. The drug-related safety profiles were consistent with those previously reported and new safety issues have not been observed after 12 months. Most drug-related AEs have developed within 12 months, and have shown minimal increase compared with rates at 12 months follow-up. Estimated OS and PFS at 36 months were 87.6% and 85.7%, respectively.

Figure 1. Summary/Conclusions: The 36 months data supports radotinib treatment in TKI failed CP-CML patients maintains the effective response and high rates of OS & PFS rate. Thus, radotinib demonstrated a promising alternative treatment for patients with TKIs failure.
100 YEARS OF CHRONIC MYELOID LEUKEMIA PREVALENCE IN FRANCE M. Delord 1,2

Background: The success of imatinib and other rationally designed tyrosine kinase inhibitors (TKIs) in the treatment of chronic myeloid leukemia has drastically improved the fate of CML patients. From a couple of years in median survival in the 70s, a majority of patient now achieve a near normal life expectancy with lifelong treatment though. This new paradigm where CML can be regarded as a chronic disease has mechanical consequences on the disease prevalence and overall costs for health care systems. The success of imatinib and other rationally designed tyrosine kinase inhibitors (TKI) in the treatment of chronic myeloid leukemia has drastically improved the fate of CML patients. From a couple of years in median survival in the 70s, a majority of patient now achieve a near normal life expectancy with lifelong treatment though. This new paradigm where CML can be regarded as a chronic disease has mechanical consequences on the disease prevalence and overall costs for health care systems.

Aims: We present here a fully detailed and comprehensive analysis of the French CML prevalence over a century from 1960 to 2060.

Methods: Prevalence of CML was estimated using a cohort component-based model frormesimates of incidence rate of the disease by age and sex, population and demo-graphic projection from the National Institute of statistics and Econo-mics Studies, and various hypotheses on the relative survival of CML patients. Prevalence of CML was estimated using a cohort component-based model from estimates of incidence rate of the disease by age and sex, population and demographic projection from the National Institute of statistics and Economics Studies, and various hypotheses on the relative survival of CML patients. The number of CML patients is estimated over time and the resulting CML prevalence expressed as a number of CML patients per 100,000 inhabitants.

Results: The CML prevalence in France, expressed in cases per 100,000 inhabitants, was estimated to be around 3 before the 80s, 6 before the 2002, 17 in 2016, 25 in 2030 where the tendency inflects, and 30 after 2040. Consid-ering the 100% relative survival hypothesis, a target CML prevalence were defined, the level of which will be nearly reached by 2060. By simulation, we showed that given constant incidence rates and high relative survival hypotheses, the CML prevalence will be driven by population aging, and that the target prevalence, defined as the maximum CML prevalence, should be nearly reached by 2050 to levels above 30 per 100,000 inhabitants.

Summary/Conclusions: Due to high rates of relative survival observed after introduction of imatinib, the trajectory of the CML prevalence in France, as in other western countries, has changed. Given particular hypothesis on the CML incidence rates, this trajectory will bring the CML prevalence by the mid century to levels fully determined by population aging. For France, we have estimated this level above 30 cases per 100,000 inhabitant. Due to high rates of relative survival observed after introduction of imatinib, the trajectory of the CML prevalence in France, as in other western countries, has changed. Given particular hypothesis on the CML incidence rates, this trajectory will bring the CML prevalence by the mid century to levels fully determined by population aging. For France, we have estimated this level above 30 cases per 100,000 inhabitant.
(SNPs) affect the transporter activity, but their impact on clinical response to imatinib in chronic myeloid leukemia (CML) is discordant; even less is known on their role in patients treated with second generation (2G) TKIs dasatinib and nilotinib.

**Aims:** To investigate the role of the most common ABCB1 and ABCG2 genetic polymorphism in chronic phase CML patients treated with imatinib and 2G-TKIs.

**Methods:** We analysed four polymorphisms of ABCB1 (129T>C, 1236C>T, 2677G>T/A and 3435C>T) and two polymorphisms of ABCG2 (34G>A and 421C>A) in 196 CP-CML patients, of whom 139 treated with imatinib (114 in first line and 25 after interferon failure) and 57 treated with dasatinib or nilotinib (22 in first line and 35 after imatinib failure). We compared the rates of optimal response at 3 months (defined as BCR/ABL <10%), at 6 months (BCR/ABL <1%) and at 12 months (BCR/ABL <0.1%), progression-free survival (PFS) and time to treatment failure (TTF) according to the different protein genotypes. TTF was calculated from the start of therapy to any of the followings: progression to accelerated or blastic phase (ABP), death, or any cause at any time, treatment discontinuation for primary or secondary resistance or intolerance. PFS was calculated from the start of TKI to ABP or death.

**Results:** A total of 196 patients with CP-CML (median age 57 years, range 21-84) were included in the analysis. Frequency of ABCB1 and ABCG2 SNPs expression is summarized in Table 1. Considering response to therapy, either in imatinib-treated patients and in those receiving a 2G-TKI, we did not find any significant difference in terms of optimal response at the various timepoints, TTF or PFS for ABCB1 C1236T, G2677T and C3435T and of ABCG2 G34A and C412A polymorphism, even if there was a trend for a worse PFS in the few patients (n=3) with 1236 allele A treated with imatinib. Conversely, we found a lower rate of optimal response at 3 (p=0.01), 6 (P=0.05) and 12 (p=0.02) months in imatinib-treated patients with TC genotype of ABCB1 T129 SNP, though the small number of patients (7) had probably impact on statistical significance. However, TTF was shorter for ABCB1 129T>C patients, both receiving imatinib (P=0.05) and 2G-TKIs (P=0.07), and also PFS was significantly shorter in this cohort (P=0.003).

**Summary/Conclusions:** With the limits of the low expression rates of some SNPs, our data suggest a lower response in patients harboring 129T>C polymorphism, at least in those receiving imatinib. Other ABCB1 and ABCG2 genotypes do not seem to impact on response to TKI treatment.

**E1066**

**THE INTRODUCTION OF SECOND-GENERATION TYROSINE KINASE INHIBITORS MAY REDUCE THE PROGNOSTIC IMPACT OF HIGH-RISK PATIENTS TO EUROPEAN TREATMENT AND OUTCOME STUDY (EUTOS) SCORE**


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**Background:** The discovery of tyrosine kinase inhibitor (TKI) imatinib has revolutionized the concept of chronic myeloid leukemia (CML) as a mortal disease to a long-term controllable disease. The European Treatment and Outcome Study (EUTOS) score is a clinical tool that utilizes imatinib-based objectives to predict treatment response and progression free survival (PFS) in patients with CML in chronic phase (CP). However, it is currently unknown whether the introduction of second generation TKIs (2nd TKIs) affects prognostic score of patients with CML-CP, particularly among those considered high-risk according to EUTOS score.

**Aims:** Our study aims to highlight the critical role of the introduction of 2nd TKIs on patient prognosis as determined by EUTOS score.

**Methods:** Patients data was obtained retrospectively from patients enrolled in the CML Cooperative Study Group. Patients with CML-CP who were treated with any TKIs as first line therapy between April 2001 and January 2016 were enrolled in the study and were classified according to date of diagnosis. Those who were diagnosed with CML-CP before March 2009 were classified into the imatinib group, and those diagnosed after April 2009 were classified into the 2nd TKI group. The study was approved by the research ethics boards of each institution and was conducted in accordance with the Declaration of Helsinki.

**Results:** There were 308 patients newly diagnosed with CML-CP during the study period. Of these patients, 104 (34%) were assigned to the imatinib group and 204 (67%) were assigned to the 2nd TKI group. With respect to EUTOS score, 223 patients were classified as low-risk, of which 69 were in the imatinib group and 154 were in the 2nd TKI group. Forty-six patients were considered high-risk, of which 19 were in the imatinib group and 27 were in the 2nd TKI group. EUTOS score was unavailable in 39 patients. With regard to initial TKI, all patients were treated with imatinib in the imatinib group. Among patients assigned to the 2nd TKI group, 149 (73%) were initially treated with any TKI. The median follow-up period for all patients was 48 months (range: 1–185 months). Among patients in the 2nd TKI group, CML-associated death rates were significantly lower than those in the imatinib group. EUTOS high-risk patients score exhibited significantly worse outcomes in EFS, PFS, and CML-associated death compared to those considered low-risk. Most importantly, risk stratification by EUTOS score was predictive of risk-associated clinical outcomes in patients assigned to the imatinib group; however, EUTOS score failed to predict risk-associated clinical outcomes of patients assigned to the 2nd TKI group (see Figure). The EUTOS high-risk patients in the imatinib group showed worse clinical outcomes than those in the 2nd TKI group (hazard ratio [HR] 6.35, 95% confidence interval [CI] 1.79 – 22.6, p=0.0042). However, prognostic effect was less in the 2nd TKI group (HR 3.21, 95% CI 0.59 – 17.6, p=0.18). Out of 308 patients, 9 progressed to AP/BC, of which 8 transformed during imatinib therapy and 1 transformed during dasatinib therapy.

**Summary/Conclusions:** Among patients assigned to the imatinib group, risk stratification by EUTOS score was predictive of clinical outcomes in that those considered high-risk experienced considerably more adverse events (EFS, PFS, or CML-associated death) than those considered low-risk. Our results support the use of 2nd TKIs in treating high-risk patients with CML-CP in order to avoid disease progression. Future large-scale studies are necessary to evaluate the clinical significance of EUTOS scoring in the accurate prediction of prognosis among patients with CML-CP treated with 2nd TKIs.
CHRONIC MYELOID LEUKEMIA DIAGNOSED DURING PREGNANCY: THERAPY TACTICS AND OUTCOMES

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Background: Chronic myeloid leukemia (CML) diagnosed at pregnancy is a serious challenge. Treatment by tyrosine kinase inhibitors (TKI) today is considered harmful for fetus due to possible teratogenicity. On the other hand TKI delay is dangerous for disease progression as no other options have comparable to TKI effectiveness. Pregnancy termination by abortion may be crucial for desired pregnancies as further childbirth is postponed for years until stable deep molecular response (DMR). Due to limited number of cases and ethical issues there is no consensus on how to behave in such delicate cases.

Aims: To describe pregnancy outcomes and therapy tactics for CML diagnosed at pregnancy.

Methods: Information regarding CML diagnosed at pregnancy was collected with the participation of physicians participating in the observational study of European LeukemiaNet (ELN Pregnancy Registry). The data included CML clinical characteristics at diagnosis, cytogenetic and molecular parameters, information of therapy, pregnancy outcomes and data of newborns.

Table 1.

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<tr>
<th>THERAPY TACTICS AND OUTCOMES</th>
<th>CHRONIC MYELOID LEUKEMIA DIAGNOSED DURING PREGNANCY</th>
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Summary/Conclusions: There is the first report of a large database of women diagnosed with CML during pregnancy. Management of this very delicate subset of patients is a challenge especially when a woman refuses from abortion. Individual treatment approach may differ considering pregnancy terms and clinical status. Although normal childbirth is possible using IM after 2nd, 3rd trimester, the risks of pregnancy prolongation remain still not well defined. To get the most safe prognosis for mother and child pregnancy in CML should be planned in a stable DMR.

RESULTS: Thirty one women with median age 26 years (range 20-39) were diagnosed with Ph-positive chronic phase CML during pregnancy. That was 11% of all 282 pregnancy cases. In certain countries (Russia) up to 21% of women with CML were diagnosed with Ph-positive chronic phase CML during pregnancy. Management of this very delicate subset of patients is a challenge especially when a woman refuses from abortion. Individual treatment approach may differ considering pregnancy terms and clinical status. Although normal childbirth is possible using IM after 2nd, 3rd trimester, the risks of pregnancy prolongation remain not well defined. To get the most safe prognosis for mother and child pregnancy in CML should be planned in a stable DMR.

IMPACT OF KIR3DL1*00501 IN TYROSINE KINASE INHIBITOR-TREATED CML


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Background: The BCR-ABL1 tyrosine kinase inhibitors (TKIs) dramatically improved long-term survival of the patients with chronic myeloid leukemia (CML). As increased NK cells during TKI therapy positively correlate with better outcomes, immunotherapy may be a new approach. Our previous data demonstrated that two factors, sex and IM resistance, strongly associate with better survival. Now, we aim to identify the role of several KIR alleles in the response to TKIs. Therefore, we performed genotyping of KIR and HLA with deep sequencing in CML patients, and report their clinical impacts.

Methods: KIR and HLA high resolution typing were performed on peripheral blood DNA from 76 CML patients in chronic phase (CML-CP) using the Scisco Genetics typing kit (Scisco Genetics Inc., Seattle WA) and MiSeq as platform by NGS. Therapeutic effects of TKIs were evaluated based on bcr-abl mRNA levels measured by real-time quantitative (RQ)-PCR compensated according to international scale (IS) and/or transcription mediated amplification (TMA) method. Major molecular response (MMR) was defined as 3-log reduction (MR3) in RQ-PCR (IS) or BCR-ABL transcript level of less than 50 copies/μg RNA in TMA method. We also defined DMR as 4-log reduction (MR4) in RQ-PCR (IS), which is similar to undetectable of BCR-ABL transcript level in TMA method. The Cox proportional hazards model was used in the time-to-event analysis with a p-value<0.05 considered statistically significant.

Results: Second generation TKIs as first-line therapy (n=46) and female (n=29) sex were strongly associated with superior DMR at the 2-year of therapy (second generation TKIs as first-line treatment, HR 7.305, 95% CI 3.770 to 15.803, p<0.001); female sex, HR, 1.709, 95% CI, 1.028 to 2.842, p<0.039). After adjustment with these two factors, several KIR alleles positively correlates with superior DMR at the 2-year; KIR2DL4*008 or 011/00501 (HR 1.942, 95%CI 1.160 to 3.250, p<0.012); KIR2DS4*00301 or 007/010 or 015 than 1.590 to 4.968, p<0.001); KIR3DL1*00501 (HR 3.634, 95% CI 1.884 to 7.013, p<0.001). Interestingly, KIR3DL1*00501 for the patients has more strong link-age to KIR2DL4*008 or 011/00501, and 2DS4*00301 or 007/010 or 015 than other KIR3DL1 alleles. (Fisher’s exact test, p<0.001).

Summary/Conclusions: KIR3DL1*00501 and several KIR2DL4 and 2DS4 alleles positively correlate with better therapeutic effects of TKIs, and they may be form the same KIR haplotype. Our data indicate that these KIR alleles represent strong anti-CML immunity by NK cells, and consequently may associate with long-term outcome and treatment-free remission in CML.

COMPARISON OF MOLECULAR KINETICS AFTER THE FIRST AND SECOND IMATINIB DISCONTINUATION: RESULTS FROM THE KID STUDY


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Background: Recent reports have demonstrated that tyrosine kinase inhibitors (TKIs) discontinuation can be employed in chronic phase chronic myeloid leukemia (CP CML) patients with sustained deep molecular responses after enough TKI therapy. Consequently, treatment-free remission (TFR) has been a new therapeutic goal. Although 50–70% of patients experienced molecular relapse by several imatinib (IM) discontinuation studies, the most of patients resumed molecular responses (MR) following restart of IM. Aim: We performed radotinib (n=11) and imatinib pulse therapy (n=10) in CML patients (Korean Imatinib Discontinuation Study; KID Study), we have explored molecular kinetics after the first IM discontinuation and after IM resumption for molecular relapse. In patients regaining durable UMRD with IM resumption, we tried second IM discontinuation and compared molecular kinetics between the first IM stop and second IM stop. Methods: CP CML patients who were treated with IM for more than 3 years and had undetectable levels of BCR-ABL1 transcripts determined by quantitative reverse transcriptase polymerase chain reaction (PCR) for at least 2 years were eligible for KID study and in cases of MMR loss after 2 consecutive assessments, IM treatment was re-introduced. After IM resumption for MMR loss, every 3 months IM was evaluated. Until until MMR was re-achieved and every 3 months thereafter. The second stop was permitted in the patients who were in second UMRD for at least 2 years.

Results: Among patients who lost MMR in 2 consecutive analyses and resumed IM in the KID study, 12 patients (8 men and 6 women) with a median age of 45 years (range, 25-59 years) entered into a second IM discontinuation after maintaining UMRD at least 2 years. Prior to first discontinuation, the median duration of IM therapy was 68.9 months (range, 38.5-115.1 months) and the duration of sustained UMRD was 32.9 months (range, 24.8-64.5 months). After first attempt of IM discontinuation, they relapsed after a median duration of 3.7 months (range, 1.0-7.7 months) after maintaining UMRD; the median duration of sustained UMRD was 6.7 months (range, 3.3-13.6 months) after IM resumption. After sustaining a second UMRD for a median of 25.5 months, IM therapy discontinued for a second time. After a median follow-up of 8.8 months (range, 0.3-38.1 months) after second IM discontinuation, 10/12 patients (83%) and 8/12 patients (67%) lost UMRD and MMR, respectively. Among patients who did not lose UMRD but lost MMR, one patient showed fluctuation of BCR-ABL1 transcript under the level of 0.1% on IS for 9.4 months and another patient have shown gradually increasing BCR-ABL1 transcripts under the level of 0.1%. Eight patients who experienced second relapse (MMR loss) after a median 2.9 months (range, 1.9-30.7 months). The patients who lost MMR, except one patient, were retreated with IM for a median of 7.1 months (range, 0.8-24.8 months); five patients re-achieved MMR at a median of 1.8 months (range, 1.0-10.2 months) and one re-achieved UMRD at 5.5 months.

Summary/Conclusions: The present study demonstrates that a second attempt might be possible and the median time to MMR loss after second discontinuation was similar to those of the first discontinuation. But the molecular kinetics after second IM resumption needs longer follow-up with more patients. Further studies on the predictors to select patients for a trial of second TFR and novel strategies such as intermittent therapy will be warranted.

E1070

CLINICAL IMPACT BY 24 MONTHS ACCORDING TO BCR-ABL1 TRANSCRIPT LEVEL AT 3 AND 6 MONTHS IN NEWLY DIAGNOSED CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH RADOTINIB 300MG BD OR IMATINIB

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Background: BCR-ABL1 transcripts ≤1% after 6 months was an effective predictor. Also, radotinib showed that optimal molecular responses at landmark both 6 months and 12 months were significantly higher than imatinib group. Aims: We evaluated the impact of molecular response by 24 months for molecular level after 3 months (BCR-ABL1 transcript level ≤1%) and 6 months (BCR-ABL1 transcript level ≤1%) of radotinib 300mg twice daily (bid) approved for first-line use in Korea or imatinib treatment in newly diagnosed CML-CP. Methods: Baseline demographics and clinical risk score; 241 patients were randomized 1:1:1 to radotinib 300mg bid (n=79), radotinib 400mg bid (n=81), or imatinib 400mg once daily (qd) (n=81). 157 patients with available 3 months qRT-PCR on study therapy [radotinib 300mg bid (n=79), and imatinib 400mg qd (n=78)] were evaluated. And, total of 151 patients who received radotinib 400mg bid (n=78) were evaluated. After maintaining UMRD at least 2 years. Molecular response was assessed by RQ-PCR at baseline and every 3 months. BCR-ABL1 transcript level was measured by RQ-PCR, standardized according to international scale (BCR-ABL1S). Major molecular response (MRR) was defined by BCR-ABL1/ABL1 ratio ≤0.1% and MMR was defined as ≥4.5 log reduction in BCR-ABL1 transcript levels from standardized baseline or BCR-ABL1/ABL1 ratio ≤0.0032%.

Results: In two study groups, early molecular response (EMR) at 3 months were observed in 86.1% of patients in the radotinib 300mg bid group and 67.9% in the imatinib group (P=0.0179). More patients treated with radotinib 300mg bid who had EMR at 3 months achieved MMR and MR4.5 by 24 months: 73.5% and 38.2% in the radotinib 300mg bid group and 63.6% and 29.1% in the imatinib group, respectively. At 6 months, 73.4% of patients in the radotinib 300mg and 53.1% patients in imatinib group (P=0.0246) achieved 6 months EMR. The patients who had EMR at 6 months in radotinib 300mg bid group were significant higher in IM and MMR compared with the other groups. More patients did not achieved MMR (P=0.0001) which they achieved MMR and MR4.5 by 24 months: 86.2% and 44.8%, respectively and imatinib group achieved 81.4% and 39.5%, respectively. By 24 months, overall survival (OS) and progression-free survival (PFS) according to 3 months or 6 months were not significantly different in two groups.

Summary/Conclusions: With minimum 24 months follow-up, early responses at 3 months or 6 months can predict better outcomes in newly diagnosed chronic myeloid leukemia patients treated with radotinib or imatinib. But, to evaluate the significant long-term prognostic value such as overall survival and progression-free survival by EMR, longer follow-up are needed.
HU+ponatinib for 72 hours, and the percentage of viable cells in each sub-clone was analyzed by flow cytometry.

**Results:** HU treatment resulted in WBC stabilization in 3 of 4 patients, but failed to induce a molecular response. However, surprisingly, the percentage of BCR-ABL1 decreased significantly in all 4 patients during HU treatment and was no longer detectable in 3 of 4 cases. Stem cell transplantation could be performed in 2 patients after 2-3 months. In one patient, stable disease over 18 months was obtained with HU-therapy. In one patient, the disease progressed rapidly despite temporary suppression of BCR-ABL1. In vitro studies, HU was found to block the growth in all cell lines tested and in all primary cell samples (n=7) examined, with IC50 values ranging between 50 and 250 µM. Interestingly, cell lines exhibiting mutant BCR-ABL1 were more sensitive against HU than cells lines expressing BCR-ABL1. HU and ponatinib were found to synergize in inhibiting growth of all cell lines tested, including cells expressing BCR-ABL1 or T315I-including compound mutations. Cooperative drug effects were also confirmed in primary CML cells (n=4). In cell line experiments, ponatinib was found to suppress Ba/F3/p210WT cells, but not Ba/F3/p210WT cells treated with HU. HU and ponatinib combination resulted in complete suppression of all sub-clones.

**Summary/Conclusions:** Our data show that HU exerts strong, sub-clone specific, specific, anti-neoplastic effects in TKI-resistant CML cells. Clinical studies are now warranted to define the exact value of the drug combination ponatinib+HU in TKI resistant CML.

**E1072**

ASSOCIATION OF BCL2L11 (BIM) DELETION POLYMORPHISM WITH MOLECULAR RELAPSE AFTER TYROSINE KINASE INHIBITOR CESSATION IN CHRONIC MYELOID LEUKEMIA PATIENTS WITH DEEP MOLECULAR RESPONSE

**Background:** The inhibition of BCR-ABL1 kinase with tyrosine kinase inhibitors (TKIs) has markedly improved the prognosis of chronic myeloid leukemia (CML). Recently, it has been recognized that some CML patients with deep molecular response (DMR) can maintain treatment-free remission (TFR) after TKI cessation. However, no predictive prognostic factor for successful treatment cessation has yet been identified. BCL2L11 (BIM) deletion polymorphism (intron 2) has been reported to be associated with an inferior response to TKI (Ng et al. Nature Medicine, 2012). We have previously reported that BCR-ABL1 cells in vivo and in vitro may predict relapse after TKI discontinuation (Katagiri et al. Br J Haematol, 2013).

**Aims:** To further clarify the role of predictive biomarkers in molecular relapse after TKI cessation, we performed a long-term follow-up of CML patients with DMR after TKI discontinuation.

**Methods:** Patients with DMR receiving TKI treatment were included. Molecular relapse was defined as a loss of the major molecular response (MMR). The genomic DNA of patients was obtained from their whole blood samples using the EZ1 DNA Blood 350 kit (Qiagen, Valencia, USA). Deletion polymorphism was detected by Q-Invader assay using primers designed to detect a deletion site (2,903 bp) (Ohyashiki et al., J Hematol Transfus, 2014).

**Results:** Forty-six CML patients (29 men; 17 women; median age, 58.5 years) were included in this study (Sokal category: low: 32, intermediate: 10, and high: 4). Thirty-three patients discontinued imatinib, five discontinued nilotinib, and eight discontinued dasatinib. Ten patients were treated with IFNα before TKI treatment. The median duration from TKI initiation to cessation was 85.0 months (range: 22–177 months); the median duration of DMR before TKI cessation was 43.0 months (range: 5–131 months). Treatment-free remission was estimated to be 66.5% at 12 months, 61.5% at 24 months, and 58.5% at 36 months. Thirty-six CML patients were analyzed for the presence of BIM deletion polymorphism and six exhibited BIM deletion polymorphism. All patients with BIM deletion polymorphism relapsed within 12 months after TKI cessation. A significant difference was observed only in BIM deletion polymorphism between the patients who maintained and those who lost MMR (p=0.0000528). No patient died during the follow-up period. No significant difference was observed in the sequelae of TKI therapy, prior IFNα therapy, and time to DMR between relapsing and non-relapsing patients.

**Summary/Conclusions:** The analysis of BIM deletion polymorphism in CML patients is expected to be useful for predicting their early molecular relapse after TKI treatment discontinuation.
Enzymopathies, membranopathies and other anemias

E1074
IDENTIFICATION OF INCIDENTS CASES OF GAUCHER DISEASE IN SPLENOMEGALY AND/ OR THROMBOCYTOPENIA PATIENTS IN SPECIALIZED MEDICAL SERVICES IN COLOMBIA THROUGH THE USE OF A SELECTION ALGORITHM


Background: Gaucher disease (GD) varies greatly in severity and organ involvement. Clinical characteristics are usually nonspecific and lead to late diagnosis with irreversible complications. Splenomegaly and thrombocytopenia are the two most common manifestations (Gaucher Registry, 2008), which reported 86% cases with moderate to severe splenomegaly and 60% thrombocytopenia at the time of diagnosis, thus demonstrating why patients are referred to hematology. A diagnosis of GD is considered after other diagnostic hypotheses have been ruled out. The consensus of international experts on GD management is coordinated by Caimed Colombia.

Aims: To identify new cases of GD in a selected population with splenomegaly and/or thrombocytopenia referred to hematology. A diagnosis of Gaucher Disease is considered after other diagnostic hypotheses (in children) or with other enzymes (in adults) presented more impact on oxidative modifications than the lower values were obtained for pairs that included CAT inhibition; when only one enzyme was inhibited, GPx inhibition showed the highest TAS. Regarding LPO, a trend towards increasing values with enzyme inhibition was observed; the lowest value was obtained when all enzymes were active, and the highest when all of them were inhibited; when only one enzyme was inhibited, CAT inhibition showed the highest LPO value and when two enzymes were inhibited, LPO was increased for the pairs that included GPx. MBH was increased for all enzyme inhibitory conditions, when compared to the condition with all enzymes active, excepting when CAT was inhibited.

Summary/Conclusions: In conclusion, inhibition of these antioxidant enzymes, either alone or simultaneously, leads to oxidative stress modifications within the RBC, as shown by the increase in MBH and membrane LPO, and by the decrease in cytosolic TAS. Moreover, the inhibition of CAT or GPx (either alone or with other enzymes) presented more impact on oxidative modifications than Prx2 inhibition. Our data strengthens the importance of these enzymes in RBC’s defense being known, the interplay between these peroxidases is still unclear. Peroxiredoxin 2, glutathione peroxidase and catalase inhibition on oxidative stress modifications of red blood cell membrane and cytosol

D. Melo1, S. Ribeiro2, A. Santos-Silva3, S. Rocha2

Methods: We performed in vitro assays (n=3) with RBCs from healthy volunteers, inhibiting Prx2, GPx and CAT, either individually, two by two or all three; conodin A, mercaptosuccinic acid and sodium azide were used as specific inhibitors of Prx2, GPx and CAT, respectively. Since the RBC membrane is a major target of ROS, we evaluated membrane lipoperoxidation (LPO) and membrane bound haemoglobin (MBH), as well as, cytosol’s total antioxidant status (TAS), by spectrophotometric methods.

Results: Concerning TAS we found a trend towards decreasing values with enzyme inhibition (one or more); the lowest value of TAS was observed when all three enzymes were inhibited and, when only two enzymes were inhibited, the lower values were obtained for pairs that included CAT inhibition; when only one enzyme was inhibited, GPx inhibition showed the highest TAS; according LPO, a trend towards increasing values with enzyme inhibition was observed; the lowest value was obtained when all enzymes were active, and the highest when all of them were inhibited; when only one enzyme was inhibited, CAT inhibition showed the highest LPO value and when two enzymes were inhibited, LPO was increased for the pairs that included GPx. MBH was increased for all enzyme inhibitory conditions, when compared to the condition with all enzymes active, excepting when CAT was inhibited.

Acknowledgements: This study was funded by Sanofi Genzyme Colombia and coordinated by Caimed Colombia.

E1075
IMPACT OF PEROXIREDOXIN 2, GLUTATHIONE PEROXIDASE AND CATALASE INHIBITION ON OXIDATIVE STRESS MODIFICATIONS OF RED BLOOD CELL MEMBRANE AND CYTOSOL

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Background: Several anemias are associated with oxidative stress, namely, sickle cell anemia, β-thalassemia, glucose-6-phosphate dehydrogenase deficiency and hereditary spherocytosis. Red blood cells (RBC) are continuously exposed to oxidative stress, mainly due to their primary function as oxygen carriers; therefore, the erythrocytes are equipped with an efficient antioxidant system, however, when its capacity is overwhelmed, the cell is exposed to reactive oxygen species (ROS), triggering oxidative modifications. The antioxidant system includes several enzymes, such as peroxiredoxin 2 (Prx2), glutathione peroxidase (GPx) and catalase (CAT); in spite of their roles in cell defense being known, the interplay between these peroxidases is still unclear. The recent report of conodin A, as a specific Prx2 inhibitor, offers the possibility to further explore the roles and contribution of these enzymes to antioxidant defense.

Aims: We aimed to study the importance of Prx2, GPx and CAT inhibition on defense against oxidative stress in normal erythrocytes.

Methods: We performed in vitro assays (n=3) with RBCs from healthy volunteers, inhibiting Prx2, GPx and CAT, either individually, two by two or all three; conodin A, mercaptosuccinic acid and sodium azide were used as specific inhibitors of Prx2, GPx and CAT, respectively. Since the RBC membrane is a major target of ROS, we evaluated membrane lipoperoxidation (LPO) and membrane bound haemoglobin (MBH), as well as, cytosol’s total antioxidant status (TAS), by spectrophotometric methods.

Results: Concerning TAS we found a trend towards decreasing values with enzyme inhibition (one or more); the lowest value of TAS was observed when all three enzymes were inhibited and, when only two enzymes were inhibited, the lower values were obtained for pairs that included CAT inhibition; when only one enzyme was inhibited, GPx inhibition showed the highest TAS; regarding LPO, a trend towards increasing values with enzyme inhibition was observed; the lowest value was obtained when all enzymes were active, and the highest when all of them were inhibited; when only one enzyme was inhibited, CAT inhibition showed the highest LPO value and when two enzymes were inhibited, LPO was increased for the pairs that included GPx. MBH was increased for all enzyme inhibitory conditions, when compared to the condition with all enzymes active, excepting when CAT was inhibited.

Summary/Conclusions: In conclusion, inhibition of these antioxidant enzymes, either alone or simultaneously, leads to oxidative stress modifications within the RBC, as shown by the increase in MBH and membrane LPO, and by the decrease in cytosolic TAS. Moreover, the inhibition of CAT or GPx (either alone or with other enzymes) presented more impact on oxidative modifications than Prx2 inhibition. Our data strengthens the importance of these enzymes in RBC’s defense being known, the interplay between these peroxidases is still unclear. Peroxiredoxin 2, glutathione peroxidase and catalase inhibition on oxidative stress modifications of red blood cell membrane and cytosol

D. Melo1, S. Ribeiro2, A. Santos-Silva3, S. Rocha2

Figure 1.
antioxidant homeostasis, and suggests that inhibition or injury to one (or more) compromises erythrocytes, which might influence clinical presentation in oxidative stress associated anemias.

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E1076
MOLECULAR BASIS OF PKLR MUTATIONS IN PATIENTS WITH PYRUVATE KINASE (PK) DEFICIENCY: THE FIRST REPORT FROM SOUTHEAST ASIAN POPULATION
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Background: Recently we have identified a new form of transfusion dependent hemolytic anemia due to KLF1 mutations causing a trans-acting deactivation of pyruvate kinase genes (PKLR). Mutations of PKLR per se can affect red blood cells metabolism and cause a wide range of clinical manifestation from fetal anemia leading to hydropic fetus, severe neonatal jaundice requiring multiple exchange blood transfusions, chronic to fully compensated hemolytic anemia. Understanding of the molecular basis of pyruvate kinase deficiency (PK def.) might be useful to predict clinical phenotypes and suggest appropriate clinical management of future patients. Moreover, an interaction of PKLR and KLF1 mutations in such patient has not been explored.

Aims: This study aim to identify the mutation of patients with PK def. for the first time in Southeast Asian populations.

Methods: Seven unrelated patients; 6 from Thailand and 1 from Indonesia have been enrolled after informed consent. We have measured the PK activity of all patients and their parents and siblings using a standard biochemical technique as we have described earlier. A complete genomic analysis of all PKLR’s exons (NM_000298.5) including exon-intron boundaries were selectively amplified and followed by direct Sanger sequencing.

Table 1.

Results: Seven index PK def. patients as confirmed by enzyme activities, age range 9-35 yrs old, were identified (Table 1). Three patients presented with severe hemolytic anemia and required regular blood transfusion; every 3-4 weeks in two (PK-1 and PK-3) and every 10-12 weeks (PK-2) in which one patient (PK-1) has been successfully treated with bone marrow transplantation and became transfusion-free. Three patients (PK-5, -6 and -8) had moderately severe hemolytic anemia and required blood transfusion occasionally. Only one patient (PK-7) from Indonesia had well-compensated anemia and never required blood transfusion. All but one had PK activities lower than 50% of normal range. In the patient (PK-4) with the lowest PK activity, we did not find any mutation in KLF1 of this patient suggesting that she might have other unidentified cis mutation involved gene regulation of KLF1. Due to a limited number of patients, there was no clear genotype-phenotype correlation found in our studied population.

Summary/Conclusions: Seven confirmed cases of PK def. are reported here-in. They showed a wide variation of clinical severity. Molecular basis of PKLR mutations was proven to be beneficial to provide a definitive diagnosis of PK def. and might help suggesting clinical presentation in future cases.

E1077
PRELIMINARY RESULTS OF GAU-PED STUDY: PREVALENCE OF GAUCHER DISEASE IN PAEDIATRIC PATIENTS SELECTED BY AN APPROPRIATE DIAGNOSTIC ALGORITHM
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Background: Gaucher disease (GD) is an autosomal recessive lysosomal storage disease characterized by the deficient activity of beta-glucocerebrosidase (GBA). GBA deficiency results in the accumulation of glucosylceramide in different organs, causing tissue damage. Typical GD features are splenomegaly, peripheral blood cytopenias (mostly thrombocytopenia and/or anemia), growth retardation, bone involvement, gammapathies, increased risk of malignancies and, in some patients, neurological manifestations. Since symptoms are non-specific, the diagnosis can be delayed for years or missed. Enzyme replacement therapy (ERT) with recombinant β-glucocerebrosidase is safe and effective in preventing and/or reversing many clinical manifestations. However, if the diagnosis is delayed for years, major complications cannot be reversed. A useful screening method for GD is based on measuring enzyme activity on a Dried Blood Spot (DBS), while the gold standard test is still considered GBA activity in cellular homogenates. A pediatric algorithm has been proposed to promote timely diagnosis and early access to ERT (figure 1).

Figure 1.

Aims: Since pediatric patients with splenomegaly and cytopenias are usually referred to pediatric hematologists, we have designed the GAU-PED study to...
evaluate the prevalence of GD among children referred to the haematology paediatric units and selected according to the above mentioned diagnostic algorithm. Here, we report a preliminary analysis of GAU-PED trial.

Methods: The GAU-PED study involves 53 centers in the context of the AIEOP Study Group, the Italian clinical research consortium in paediatric haematology and oncology. Patients referring to the pediatric hematology and oncology units for diagnosis of splenomegaly with or without anaemia (thrombocytopenia, haemolytic anaemia and/or anaemia), where other causes of splenomegaly has been excluded, are tested for GBA activity through a DBS sample. Only patients with DBS showing a GBA activity below normal values are recalled to confirm GBA enzyme deficiency using the gold standard GBA analysis in cell homogenate. For every tested family clinical information are also added.

Results: After parental consent, a total of 25 DBS have been collected from 11 centers, in the first 12 months of study accrual. DBS values under 4.4 pmol/punch (n=9) were found in 9/25 patients (36%). These patients have been recalled for the conventional enzymatic test. The diagnosis of GD has been confirmed in 5/9 patients (55%), with a prevalence of GD of 20% (95% CI 8.8-39.1%) equal to 5/25 patients in the tested population. In all 5 patients the genetic analysis has been consistent with GD. Three patients were males and 2 females. The mean age at diagnosis was 8 years (range 2-13 years). The median time from the initial clinical presentation and diagnosis has been 12 months (range 6-50 months), while the mean time between the DBS test and the diagnosis has been 2 months. ERT has been started in all GD patients.

Summary/Conclusions: Our preliminary results support the use of DBS as screening test for GD in a selected population of children with splenomegaly and/or thrombocytopenia considered at increased risk for the disease. The use of a family diagnostic algorithm is useful to increase awareness of GD among pediatric hematologists and to shorten the time to diagnosis. Taking into consideration the long life expectancy of pediatric GD patients, the early diagnosis will have a strong impact on health and quality of life.

E1078 CIRCULATING MICROPARTICLES IN CONGENITAL AND ACQUIRED HAEMOLYTIC ANAEMIA W. Barcellini1,1, V.M. Sciumbata1, J.A. Giannotta1, A. Zaninoni1, V.B. Valli1, G. Merati2, E. Trombeta3, V. Ferri1, L. Petite1, A. Cortelezzi11, A. Antoni2
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Background: Microparticles (MPs) are small particles budding from cells, which contain variable amounts of proteins, miRNA and cytokines from the parental cell. MPs play a role both in physiological and pathological conditions such as signal transduction, cell activation, thrombosis and cancer. Thrombotic events are a possible complication of haemytic conditions, both congenital and acquired. Elevated levels of circulating MPs have been described in several haemytic conditions, including sickle cell anaemia, thalassemia intermedia, haemytic uremic syndrome, and thrombotic thrombocytopenic purpura. Aim: To evaluate platelet MPs (PMP), tissue factor expressing MPs (TFMPs), endothelial MPs (EMPs) and microparticles expressing single antigens (CD41, CD142 or CD142) levels in in other haemytic anaemias, such as hereditary spherocytosis (HS), elliptocytosis (HE), stomatocytosis (HST), red cell enzymatic defects, congenital dyserythropoietic anaemia (CDA), autoimmune haemytic anaemia (AIHA), and paroxysmal nocturnal haemoglobinuria (PNH).

Methods: To determine MPs, whole blood was collected into 0.109 M sodium citrated vacutainer tubes. Platelet Free Plasma (PFP) was prepared by double centrifugation at 2500 g for 15 min and stored frozen at -80°C until assayed. For MPs analysis 25 µl of PFP was incubated with annexV-APC, CD41-FITC, CD142-PE and CD144 PerCp-Cy5.5 in Hepes buffer in the presence of 15 mM CaCl2 and 1 µM of f-Hirudin for 30 min. Samples were diluted with 500 µl Annexin Binding buffer, 25 µl of fixed fluorescent dyes were added to express MP count as absolute numbers. MPs analyses were performed on a BD FACs Canto cytometer using Megamix-Plus SSC to define the MPs gate.

Results: MPs levels were evaluated in plasma of 43 patients followed-up for a median time of 9 years (range 2-34) and compared with normal controls. The median value of MPs in individuals older than 80 years (range 22-87, 9/43 (21%) had been splenectomized and 13/43 (30%) were treated at the moment of the study (steroids/immunosuppressors for AIHA, and eculizumab for PNH). Table shows Hb levels, PLT and WBC counts of the different haemytic conditions, along with LDH and reticulocyte values. In AIHA, the number of platelets was positively correlated with Hb levels (p=0.05), and MPs positively with reticulocytes and LDH (p<0.005 for both); CD41+ MPs, CD142-PE+ MPs, PMPs, TF-MPs, and EMPs positively correlated with disease duration (p<0.05). In membrane defects the following positive correlation were observed: CD41+ MPs and PMPs with platelet values and EMPs with LDH (p<0.05 for all). In PNH, annexV APC and CD41+ MPs were positively correlated with reticulocyte counts (p<0.05). In CDAs, the number of annexV APC and PMPs negatively correlated with Hb (p<0.05). Finally, the number of annexV APC was increased in PKD compared with controls (p=0.023), and positively correlated with disease duration (r=0.099, p<0.001); PMPs and TF-MPs were elevated too, although not significantly. The number of MPs here investigated were comparable between splenectomized and not splenectomized, and between naive and treated cases.

Table 1.

Summary/Conclusions: These preliminary results suggest that MPs levels are abnormal in both congenital and acquired haemytic conditions. MPs levels correlate with the degree of anaemia and haemolysis and with the duration of disease.

E1079 THE PREVALENCE, ETIOLOGY AND PROGNOSTIC IMPACT OF ANEMIA IN OLDER POPULATION S.S. Michalak1, J. Rupa-Matysyk2, L. Gif1
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Background: The population of people aged ≥60 years is growing rapidly. Anaemia represents a common condition among the elderly, however its prevalence and causes are not well known.

Aims: The aim of the study was to evaluate the prevalence, severity and etiology of anaemia in the population aged ≥60 years. Risk factors for the development of anaemia including concomitant diseases and treatment, were analysed. The association between anaemia and hospitalization or all-cause mortality during follow-up was determined.

Methods: Retrospective analysis was performed on 981 Caucasian, outpatient patients aged ≥60 in Poland over 2013-2014 (median age 68, range 60-99 years, 60% females). The prevalence of anaemia, defined according to WHO criteria, was evaluated. Age, gender, disease, host of anaemia, thrombocytopenia, anemia and etiology of anaemia were studied. Data on the incidence of common comorbidities (coronary artery disease, heart failure, diabetes, chronic obstructive pulmonary disease, chronic kidney disease, chronic liver diseases, cancer, thyroid diseases), hospitalization, treatment used and all-cause mortality were analysed.

Results: The prevalence of anaemia in the aged population was 17.2% and was higher in men than women (20.4% vs 15.2%, p=0.038). Anaemia was present in 10.3% of patients aged 60-69, in 20.1% of those aged 70-79 and in 35.6% of patients ≥ 80 years. Incidence rates of anemia increased significantly with age (60-69 vs 70-79 years, p<0.001; 60-69 vs ≥80 years, p<0.001; 70-79 vs ≥80 years, p<0.001). Anaemia was mild in 69.8% of patients, but a severe form was found significantly more often among men aged ≥80 years (p=0.03).

Analysis of the etiology of anaemia revealed three predominant types: anaemia of chronic disease (31.3%), unexplained anaemia (28.4%) and deficiency anaemia (22.5%, including iron deficiency 13%). In comparison to patients without anaemia, those with anaemia were older (p<0.001), had a higher prevalence of comorbidities (p<0.001) and were more often hospitalized (p<0.001). In the multivariate logistic regression model, factors increasing the risk of anaemia were: age ≥80 years (OR=2.29; 95%CI 1.19-4.42; p=0.013), the number of comorbidities (2 diseases OR=2.85; 95%CI 1.12-7.30; p=0.029, 3 diseases OR=6.28; 95%CI 2.22-17.76; p=0.001, 4 diseases OR=4.64; 95%CI 1.27-17.01; p=0.021) and the number of hospitalizations (OR=1.34; 95%CI 1.13-1.58; p=0.001). At the end of the 2-yr follow-up, the cumulative survival among patients without anaemia in relation to the group with anaemia was 90.76% vs 78.68% and the difference was significantly (p<0.001). In multivariate model, factors that significantly increased the risk of death in study population were anaemia (HR=3.33; 95%CI 1.43-7.74; p=0.005), cancer (HR=3.31; 95%CI 1.47-7.49; p=0.004) and heart failure (HR=2.94; 95%CI 1.33-6.51; p=0.008).

Summary/Conclusions: In patients ≥60 years the incidence of anaemia increases with age, gender and male gender, number of comorbidities and frequency of hospitalization. The high rate of unexplained anaemia indicates the necessity for detailed hematologic diagnosis. The occurrence of anaemia among people aged ≥60 years has an adverse impact on survival.

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E1080

PIEZ01 MECHANOTRANSDUCTIVE PROTEIN MUTATIONS IN RBCS: WHEN THE PHENOTYPE IS BEYOND HYALOMYCTIC ANAEMIA

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Background: Piezo proteins are integral membrane proteins with many transmembrane domains broadly expressed, including erythrocytes (RBCs). PIEZ01 proteins play an important role as an osmoreceptor, maintaining RBCs ionic homeostasis, functioning as a mechanically activated cation channels. Mutated PIEZ01 proteins have been linked to hereditary xerocytosis (HX), which is characterized by RBCs dehydration with mild to moderate compensated haemolytic anaemia and iron overload. As these clinical features are present in many different clinical conditions, the diagnosis always needs a high level of suspicion. Nowadays, besides peripheral blood smear (PBS) observation, molecular analysis, searching for mutations in PIEZ01 gene, became a tool in the diagnosis of HX.

Aims: Describe 26 patients with HX associated with PIEZ01 mutations belonging to 13 unrelated families, raising awareness of the highly variable phenotype of this patients, and the need of a high grade of suspicion along with the morphologic evaluation of the PBS.

Methods: Collection of clinical and laboratory data on our 26 patients with HX and hyperferritinaemia due to 10 different identified mutations in PIEZ01. Sanger sequencing was used to identify mutations affecting PIEZ01, encoded by FAM3B gene, and to confirm transmission according to the presence of disease phenotype. In all patients were excluded other known causes of hyperferritinaemia (HF) and haemolytic anaemia.

Results: Of the patients identified having PIEZ01 mutations, 13 were probands and 13 were identified by family studies. Median age at diagnosis was 43 years (1-80), with female predominance (n=14; 53.9%). 4/13 probands had family history of HX (n=1) or HF (n=2). The common feature of our entire cohort of patients was the presence of xeroocytes in PBS. 13/26 patients had reticulocytosis with a median reticulocyte count of 101x10^9/L (28.1-557.3), 18/26 patients had HF with a mean value of ferritin of 556ng/mL (161-6617) and 9/26 had both. Of the 26 patients, four had splenomegaly and six gallbladder lissiasis (5/6 cholecystectomized), two of them have both. Only 5 patients presented with anaemia (Hb <12g/dL), 2 macrocytic and 3 normocytic. One patient had lymphopenia. He also had an abnormal white cell carrier. We detected heterozygous missense mutations in all 26 patients.

Summary/Conclusions: HX is a dominant disorder of RBCs dehydration presenting a great phenotypic variability. As shown in our cohort of patients, the anaemia may not be the main feature, in fact, the presence of xeroocytes in PBS and HF were the most frequent characteristics of our patients. We would like to emphasise that in the genomics era the identification of xeroocytes in the PBS keeps playing an important role for this diagnostic. Not only because, unlike other haemolytic anaemias, in HX there is a contraindication to splenectomy due to the increased risk of thrombotic events, but also because this pathology presents a diagnosis that is difficult to the degree of hemolysis. This iron overload may be related to a defective iron homeostasis dependent on PIEZ01 function not strictly related with Xerocytosis.

E1081

MODELLING PYRUVATE KINASE DEFICIENCY IN HUMAN PROGENITORS USING CRISPR/CAS9

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Background: Pyruvate Kinase Deficiency (PKD) is an autosomal recessive disorder caused by mutations in the PKLR gene. PKD produces chronic non-spherocytic hemolytic anemia, which can be fatal during early childhood and may require transfusion support. Gene correction approaches, despite therapeutic splenectomy. Although not considered a standard-of-care, allogeneic bone marrow transplantation has been curative in a limited number of cases. These findings, and the evidence that PKLR gene mutations are likely the sole etiology of the disorder, make PKD a candidate for gene therapy. We developed a gene therapy strategy in a PKD mouse model using a lentiviral vector (LV) carrying a codon-optimized version of the PKLR cdNA (corPKR). This vector has been recently designated as Orphan Drug for the treatment of PKD by the EMA and FDA (EMA: EU/3/14/1330; FDA: DRU-2016-5168).

Aims: To test the efficacy of the therapeutic LV, we have proposed an alternative to pig-derived PKD-hematopoietic progenitors (PKR Progenitors). In particular, we have generated CRISPR/Cas9 system tools to knock-out the PKLR gene in healthy hematopoietic progenitors from healthy cord blood samples.

Methods: Up to six different gRNAs were specifically designed to cleave the exons 8, 9 and 11 of the PKLR gene. All gRNAs contain at least 3 mismatches with the cognate present in the therapeutic LV, to avoid the cleavage of the therapeutic transgene. Two gRNAs cleaved the PKLR gene both in 293T cells and primary CD34+ cells. In order to identify and select edited cells, Cas9-gRNAs components were cloned into a Cas9-2A-ZsGreen1 plasmid.

Results: Cord Blood CD34+ cells were electroporated, sorted and differentiated along the erythroid lineage. Significantly, the pyruvate kinase activity in ex vivo differentiated erythroid cells was impaired in gene edited cells as compared to non-edited samples.

Summary/Conclusions: Gene edit of wt CD34+ progenitors allow us to generate cells with RPKP, impaired. The decrease of PK activity validates this approach as a human model for PKD.

E1082

PHYSIOPATHOLOGY OF HEREDITARY XEROCYTOSIS : PIEZ01 GAIN OF FUNCTION MUTATIONS IMPACT HEMOGLOBIN OXYGEN AFFINITY

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Background: Dehydrated hereditary stomatocytosis, also called hereditary xerocytosis (HX) is a dominant non-spherocytic chronic hemolytic anemia characterized by an increased cation leak through the red cell membrane, associated with dehydration and shortened red cell survival. Clinically, most patients present a totally compensated hemolysis, with a normal hemoglobin level contrasting with a high reticulocytosis. In most cases, HX is caused by missense mutations activating Piezo1, a mechanosensitive ion channel. However, the pathophysiology of this compensated hemolysis remains largely unclear.

Aims: We studied the hemoglobin oxygen affinity parameters in HX patients and in hereditary spherocytosis (HS) subjects as controls.

Methods: Fourteen patients from 5 described and 4 unreported families with a HX diagnosis and 15 HS subjects were included. Diagnosis was based on electrophotometry and EMA assay. PIEZ01 and KCN4 coding regions were analyzed by Sanger sequencing in all HX patients. Hemoglobin oxygen affinity was evaluated using p50 measured on venous blood on a Hemoxanalysor or a Radiometer blood gas analyzer. 2,3 diprophosphoglycerate (2,3 DPG) levels were measured using a commercialized kit and expressed as a molar ratio 2,3 DPG/hemoglobin.

Results: All the 14 HX patients carried one or two missense mutations in PIEZ01, no gene variation was identified in KCN4. Five families (9 subjects) have already been reported, with identified mutations in exon 18, 21, 42 or 51. Five subjects from 4 new families carried new mutations in exons 14, 16 and 25 for which bioinformatic softwares showed a high likelihood of pathogenicity. For all HX patients, p50 values were under the normal range (mean 21,1, range 19,7-23,4, normal range 25-29 mmHg), contrasting with HS subjects for whom p50 was found to be in the normal range (mean 26,1, range 24,6-28,8 mmHg). This indicated a significant increase in the hemoglobin affinity for oxygen restricted to PIEZ01 mutated HX. Of note, p50 was not correlated with the Hb level (mean 139, range 112-180 g/L in HX patients versus 125, range 93-142 g/L in HS patients). Intracellular red cell 2,3 DPG level could be measured in 7 HX patients from 4 families, it was found decreased in all of them (0.43+/-0.06, normal 0.9+/-0.19), providing a pathophysiological basis for the increased hemoglobin affinity for oxygen associated to PIEZ01 mutated HX. Of note, p50 was not correlated with the Hb level (mean 139, range 112-180 g/L in HX patients versus 125, range 93-142 g/L in HS patients).

Summary/Conclusions: The increased hemoglobin affinity for oxygen observed in HX patients reflects the decrease in the 2,3 DPG level. This may be a consequence of a high ATP requirement and an increased glycolytic activity in HX red cells at the expense of the 2,3DPG shunt in order to maintain the cell ion homeostasis. High hemoglobin affinity for oxygen may induce a relative tissue hypoxia and consequently a high reticulocytosis, providing a clue to explain the compensated hemolysis frequently observed. However, the links between PIEZ01 mutations, red cell enzymatic activity and erythropoiesis need to be clarified and a proteomic and a metabolomic approach is under investigation.
Gene therapy, cellular immunotherapy and vaccination

E1083
SAFETY AND EFFICACY OF MULTI-PATHOGEN-SPECIFIC T CELLS IN A HUMANIZED MODEL OF INVASIVE ASPERGILLOSIS: A PROOF OF CONCEPT STUDY
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Background: Viral infections, most commonly by cytomegalovirus (CMV), Epstein-Barr virus (EBV), polyomavirus type I (BK), and fungal infections, mainly by Aspergillus Fumigatus (Asp), are leading causes of transplant-associated mortality in patients undergoing allogeneic hematopoietic stem cell transplantation. Standard treatment with antiviral and antifungal pharmacological agents, is often ineffective or toxic and may lead to resistance. Due to these limitations, adoptive immunotherapy with antigen-specific T cells has emerged as an attractive alternative. Towards unleashing its full potential and treat multiple viral and fungal infections by a single T-cell product, we developed a rapid, simplified and minimally laborious protocol for the generation of multipathogen-specific T cells (mp-STs) that simultaneously target CMV, EBV, BK, and Asp, from healthy donors.

Aims: Due to the lack of mouse models recapitulating the clinical condition of multiple opportunistic infections in transplanted hosts, we here aimed to test the in vivo safety of produced mp-STs and provide a proof of concept of their efficacy in a humanized model of invasive aspergillosis (IA).

Methods: mp-STs were generated from healthy donors by pulsing 1.5x10⁷ mononuclear cells with viral (CMV, IE1, pp65; EBV: EBNA1, LMP2, BZLF1; BK: Large T, VP1) and Asp peptides (Cr1f, Ge1, SHMT) and culturing for 10 days. The specificity of mp-STs was analyzed by IFN-γ Elispot. A total of 1.5x10⁷ of immunomagnetically isolated CD3+ cells (donor lymphocyte infusions-DLI) or mp-STs were infused in myelo/immuno-ablated NSG mice which had been intranasally inoculated with Asp conidia or left uninfected. Mice were evaluated by a 5-parameter sickness score and at sacrifice, tissues were assessed by histology and immunohistochemistry.

Results: We generated 23±5±1x10⁷ mp-ST cells (12-fold expansion). All cell lines were polyclonal expressing central memory markers and specific against Asp [spot forming cells (SFC)/2x10⁶ cells: 31±5±2] and the targeted viruses, if derived from seropositive donors [SFC/2x10⁶ cells, CMV: 63±7±267; EBV: 744±158; BK: 578±118]. To first address the safety issue, we asked whether mp-STs can induce acute graft-versus-host disease (aGvHD) in myelo-ablated mice. While DLI-induced mice developed clinically and histologically confirmed aGvHD and succumbed by day 20, mp- ST-mice survived free of aGvHD until the day of sacrifice (day 28). To assess the in vivo functionality of mp-STs against IA, conditioned and Asp-inoculated mice, received mp-STs (n=5), DLI (n=4) or were left untreated (IA control, n=6). All IA- and DLI-mice succumbed to histologically evidenced IA at a median day 6, whereas 60% of mp-ST-mice survived until sacrifice at day 12. The day-12 survivors presented high T-cell engraftment in the lung (%CD3+/CD45+/14±7) with no histological evidence of IA, the two mp-ST-non-survivors died from IA in the absence of T-cell engraftment. Non-specific DLI failed to control IA despite T-cell presence in 3∕4 DLI-mice (%CD3+/CD45+/spleen: 58±12, lung: 3±1) which succumbed early, before aGvHD development.

Summary/Conclusions: Overall, engrafted mp-STs effectively controlled IA without evidence of alloreactivity. Based on the robust specificity of our mp-STs against all targeted pathogens and the clinical efficacy of virus-specific T-cells, we expect that our “four in one” T-cell product has the potential to also fight the targeted viruses and become a powerful tool for the treatment of multiple, life-threatening post-transplant infections.

E1084
DONOR LYMPHOCYTE INFUSION IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES LEADS TO DIVERSITY OF LEUKEMIA-ASSOCIATED AND TIGENS-SPECIFIC T CELL RESPONSES AND TO REDUCTION IN REGULATORY T CELL FREQUENCY
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Background: Cytotoxic T-cell (CTL) responses against malignant cells play a major role in maintaining remission and prolonging overall survival in patients with hematologic malignancies after allogeneic stem cell transplantation (allo-SCT) and/or donor lymphocyte infusions (DLI). Graft versus leukemia (GvL) effects after allogeneic stem cell transplantation and/or DLI are considered to be T cell-mediated. Many groups described specific T-cell responses against several leukemia-associated antigens (LAA) in different hematological malignancies. However, T cell responses after allo-SCT and DLI are not well characterized.

Aims: In this study, we analyzed LAA-specific T cell responses after allo-SCT and DLI. To this end, we assessed the frequency and diversity of LAA-specific CD8+ T cells using ELISPOT analysis and tetramer assays in 12 patients (5 patients pts) with acute myeloid leukemia, 2 pts with chronic myeloid leukemia, 3 pts with multiple myeloma and 2 pts with chronic lymphatic leukemia) before and after DLI. Epitopes derived from PRAME, NPM1m1d, RHAMM, WT-1 and other LAA were tested. Moreover, the frequency of regulatory T (Treg) cells was measured and the course of cytokine profiles before and after DLI was analyzed. These immunological findings were correlated to the clinical course in the respective patients.

Methods: In ELISPOT and tetramer assays, an increase in frequency and diversity of LAA-specific T cells was observed in all patients. Cytokine assays using ELISA for the detection of more than 10 cytokines before and after DLI were employed.

Results: Importantly, there was a significant increase from 0 to 7 LAA-derived T cell epitopes (P<0.03) in clinical responders (R) when compared to non-responders (NR). These positive results in R versus NR where confirmed by tetramer-based flow cytometry assays, where an increase in frequency from 0.5 to 2.3% in the R group of LAA-specific T cell/all CD8+ T cells was observed. Interestingly, the frequency of Tregs in clinical responders decreased significantly from a median 72.8% to 54.6% (P=0.008) while the frequency of Tregs stayed stable over time in non-responding patients. T cell subset analysis did not reveal significant differences before versus after DLI administration. In cytokine assays using ELISA we found a significant increase of IL-4 after DLI.

Summary/Conclusions: Taken together, we detected an increase of specific CTL responses against several LAA after allogeneic stem cell transplantation and donor lymphocyte infusion. Moreover, this study suggests that broader LAA epitope-specific T cell responses as well as decreasing numbers of Tregs contribute to clinical outcome of patients treated with DLI.

E1085
GENE-MODIFIED NK-92MI CELLS EXPRESSING A CHIMERIC CD16/CD64-BB-Ζ RECEPTOR EXHIBIT ENHANCED CANCER-KILLING ABILITY IN COMBINATION WITH THERAPEUTIC ANTIBODY
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Background: Natural killer (NK) cells play a pivotal role in monoclonal antibody-mediated immunotherapy through an antibody-dependent cell-mediated cytotoxicity (ADCC) mechanism. NK-92mi is an interleukin-2 (IL-2)-independent cell line, which was derived from NK-92 cells with superior cytotoxicity to a wide range of tumor cells in vitro and in vivo. However, the Fc-receptor (CD16), which usually mediates ADCC, is absent in NK-92 and NK-92mi cells.

Methods: mm-NK-92 and mm-NK-92mi cells were infected with the lentivirus vector containing a GFP reporter gene under the control of a CMV promoter and then sorted for GFP+ cells. The gene expression efficiency was determined by flow cytometry. Then, NK-92 and NK-92mi cells were transduced with an AAVmediated vector containing the gene for a chimeric CD16/CD64-BB-ζ receptor. The expression of the transfected receptor was confirmed by immunoblotting. The killing efficiency of NK-92 and NK-92mi cells was determined by a 5-day chromium release assay using different tumor cell lines.

Results: The gene expression efficiency of mm-NK-92 and mm-NK-92mi cells was determined by flow cytometry. The results showed that the transduction efficiency of mm-NK-92 and mm-NK-92mi cells was high. The expression of the transfected receptor was confirmed by immunoblotting. The killing efficiency of NK-92 and NK-92mi cells was determined by a 5-day chromium release assay using different tumor cell lines.

Summary/Conclusions: The results of this study demonstrated that the combination of therapeutic antibodies with gene-modified NK-92mi cells expressing a chimeric CD16/CD64-BB-ζ receptor could enhance the cancer-killing ability of NK-92mi cells in vitro and in vivo.

Figure 1. NK-92miCD16 and NK-92miCD64 functional validation in vitro and characterization. A. Schematic representation of the CD16-BB-ζ and the CD64-BB-ζ receptor constructs. B. Exogenous CD16 or CD64 expression on surfaces of NK-92mi cells are shown. C. Immunoblot analysis of CD3ζ fusion protein expression in NK-92miCD16 or NK-92miCD64 cells.
Aims: To apply NK-92MI cell-based immunotherapy in cancer, we designed and generated two chimeric receptors which can bind the Fc portion of human immunoglobulins in NK-92MI cells.

Methods: The construct included the low-affinity Fc receptor CD16 (158F) or the high-affinity Fc receptor CD64, with the addition of the CD8a extracellular domain, CD28 transmembrane domains, two costimulatory domains (CD28 and 4-1BB), and the signaling domain from CD3ζ. The resulting chimeric receptors, termed CD16-BB-ζ and CD64-BB-ζ, were utilized to generate chimeric receptor-modified NK-92MI cells, which were named NK-92MIhCD16 and NK-92MIhCD64 cells, respectively.

Results: We found that NK-92MIhCD16 and NK-92MIhCD64 cells significantly improved cytotoxicity against CD20-positive non-Hodgkin’s lymphoma (NHL) cells in the presence of rituximab.

Summary/Conclusions: These results suggest that the chimeric receptor-modified NK-92MI cells could potentially enhance the clinical responses mediated by currently available anticancer monoclonal antibodies (mAbs).

E1086

A NOVEL IN VITRO METHOD TO QUANTIFY THE PHARMACOLOGICAL ACTIVITY OF BISPECIFIC ANTIBODIES IN HEMATOLOGICAL SAMPLES

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Methods: For this purpose, different fresh whole Bone Marrow (BM) or Peripheral Blood (PB) were tested with their corresponding BsAbs at 8 different concentrations in different time points (24h-144h). In this sense, we tested 31 AML BM samples (5 paired BM and PB) with the CD123XD3C (Creative Biolabs) and 7 CLL and 3 B-ALL samples with Blinatumumab (Amgen). When appropriate, baseline quantification of TAA was performed by flow cytometry (FCM). The PharmaFlow platform by FCM flow cytometry assay using the PharmaFlow platform by FCM and generated two chimeric receptors which can bind the Fc portion of human immunoglobulins in NK-92MI cells.

Aims: To apply NK-92MI cell-based immunotherapy in cancer, we designed and generated two chimeric receptors which can bind the Fc portion of human immunoglobulins in NK-92MI cells.

Methods: The construct included the low-affinity Fc receptor CD16 (158F) or the high-affinity Fc receptor CD64, with the addition of the CD8a extracellular domain, CD28 transmembrane domains, two costimulatory domains (CD28 and 4-1BB), and the signaling domain from CD3ζ. The resulting chimeric receptors, termed CD16-BB-ζ and CD64-BB-ζ, were utilized to generate chimeric receptor-modified NK-92MI cells, which were named NK-92MIhCD16 and NK-92MIhCD64 cells, respectively.

Results: We found that NK-92MIhCD16 and NK-92MIhCD64 cells significantly improved cytotoxicity against CD20-positive non-Hodgkin’s lymphoma (NHL) cells in the presence of rituximab.

Summary/Conclusions: These results suggest that the chimeric receptor-modified NK-92MI cells could potentially enhance the clinical responses mediated by currently available anticancer monoclonal antibodies (mAbs).

E1087

HUMANIZED CD7 NANOBODY-BASED IMMUNOTOXINS EXHIBIT PROMISING ANTI-T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA POTENTIAL

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Methods: Three new types of immunotoxins, dhuVHH6-PE-LR, dhuVHH6-PE-LR, and dhuVHH6-PE-LR, were successfully constructed. These recombinant immunotoxins were expressed in E. coli and showed that nanobody immunotoxins have the benefits of easy soluble expression in a prokaryotic expression system.

Results: Flow cytometry results revealed that all immunotoxins still maintained their ability to specifically bind to CD7-positive T lymphocytes and primary T-cell acute lymphoblastic leukemia (T-ALL) cells. Further in vivo animal model experiments showed that humanized dhuVHH6-PE38 immunotoxin can tolerate higher doses and extend the survival of NCG mice transplanted with CEM cells without any obvious decrease in body weight. Further studies on NCG mice model with patient-derived T-ALL cells, dhuVHH6-PE38 treatment significantly prolonged mice survival with around 40% survival improvement. However, it is also noticed that despite dhuVHH6-PE-LR showed strong anti-tumor effect in vitro, its in vivo anti-tumor efficacy is disappointed.

Background: Nanobodies, or named as VHVs, are derived from heavy-chain-only antibodies that circulate in sera of camels. Their exceptional physicochemical properties, possibility of humanization and unique antigen recognition properties make them excellent candidates for targeting delivery of biologically active components. In our previous efforts, we have successfully generated the monovalent and bivalent CD7 nanobody-based immunotoxins, which can effectively trigger the apoptosis of CD7 positive malignant cells.

Aims: To pursue the possibility of translating those immunotoxins into clinics, we humanized the nanobody sequences (designated as dhuVHH6), as well as further truncated the Pseudomonas exotoxinA (PE) derived PE38 toxin to produce a more protease-resistant form which is named as PE-LR, by deleting majority of PE domain II.

Methods: Three new types of immunotoxins, dhuVHH6-PE38, dhuVHH6-PE-LR, and dhuVHH6-PE-LR, were successfully constructed. These recombinant immunotoxins were expressed in E. coli and showed that nanobody immunotoxins have the benefits of easy soluble expression in a prokaryotic expression system.

Results: Flow cytometry results revealed that all immunotoxins still maintained their ability to specifically bind to CD7-positive T lymphocytes and primary T-cell acute lymphoblastic leukemia (T-ALL) cells. Further in vivo animal model experiments showed that humanized dhuVHH6-PE38 immunotoxin can tolerate higher doses and extend the survival of NCG mice transplanted with CEM cells without any obvious decrease in body weight. Further studies on NCG mice model with patient-derived T-ALL cells, dhuVHH6-PE38 treatment significantly prolonged mice survival with around 40% survival improvement. However, it is also noticed that despite dhuVHH6-PE-LR showed strong anti-tumor effect in vitro, its in vivo anti-tumor efficacy is disappointed.
Summary/Conclusions: We have successfully constructed a targeted CD7 molecule modified nanobody (CD7 molecule improved nanobody) immunotoxin dhuVH66-PE38 and showed its potential for treating CD7-positive malignant tumors, especially T-cell acute lymphoblastic leukemia.

**E1088**

STATINS MAY IMPROVE CAR-NK IMMUNOTHERAPY IN MM BY PREVENTING LOSS OF BCMA EXPRESSION ON MM CELLS

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Background: Chimeric Antigen Receptor (CAR) modified immune cells targeting BCMA against multiple myeloma (MM) has appeared as a feasible immunotherapy strategy to treat MM patients. However, high doses of CAR immune cells are required to achieve a response. Cord blood derived NK cells (CB-NK) is a feasible source of obtain NK cells to modify with a CAR against BCMA. We previously observed that MM cells exposed to CB-NK are able to transfer MM proteins, such as BCMA, both to CB-NK and to adjacent MM cells non-exposed to CB-NK. Furthermore, statins, which are toxic for MM cells, by altering the lipid composition of tumor cell membrane are involved in cell-cell communication. We hypothesized that statins could prevent the loss of BCMA expressed the loss of BCMA sum cells after CB-NK exposure, allowing infusing a lower CAR immune cell dose in MM patients

Aims: To evaluate the effect of statins on MM cell proliferation, on the CB-NK immune response against MM, and on BCMA expression in MM cells after CB-NK exposure

Methods: The cytotoxicity of statins against MM cells was determined in vitro and in vivo in a murine MM model; furthermore, their impact in CB-NK cytotoxicity against MM was also determined in vitro. BCMA expression on MM cells after CB-NK exposure was analyzed by confocal microscopy and by flow cytometry. FACS sorting experiments were performed to analyze BCMA transfer between CB-NK exposed MM cells to neighboring non-exposed CB-NK MM cells.

Results: Atorvastatin and Fluvastatin treatment (1µM) decreased MM cell line (ARP1, RPMI, KMM1) proliferation. No effect was detected for U266 MM cells and for K562 non-MM cells. In vivo studies, showed that mice treated for three days I.P with Fluvastatin (1mg/kg) showed significant decreased MM disease progression. Blocking of BCMA decreased CB-NK cytotoxicity against MM cells. Furthermore, pretreatment of MM cells with Fluvastatin (3 µM) increased CB-NK cytotoxicity against all MM cell lines; no impact was observed against K562 non-MM cells. Co-culture experiments showed that, as soon as 30 minutes, CB-NK exposure led to a BCMA transfer from MM cells to CB-NK and to the extra-cellular milieu leading to a loss of BCMA expression on MM cells. Fluvastatin pretreatment prevented loss of BCMA expression. After two days of co-culture, alive MM cells still showed decreased BCMA surface expression, and surprisingly, increased intracellular BCMA expression. Fluvastatin pretreatment partially avoided the MM cell exposure to CB-NK, transferred BCMA to neighboring non-CB-NK exposed MM cells which was partially inhibited with Fluvastatin pretreatment.

Summary/Conclusions: Our findings show that besides the anti-MM activity of statins alone, they avoid the loss of BCMA expression on MM cells after immune cell exposure. Preventing loss of BCMA expression on MM cells might improve the efficacy of CAR immunotherapy against BCMA, suggesting the potential of statins as an adjuvant in CAR-NK immunotherapy against MM

**E1090**

B- AND T-CELL IMMUNE REPERTOIRE PROFILING WITH ANCHORED MULTIPLEX PCR AND NEXT-GENERATION SEQUENCING

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Background: NGS-based analysis of the immune repertoire (IR) is a powerful tool to monitor disease, adaptive immune responses to disease, vaccination and therapeutic interventions. IR characterization by NGS usually requires large primer panels to cover its extensive combinatorial diversity, and a complex system of synthetic controls to account for differential amplification efficiency across segment combinations. Anchored Multiplex PCR (AMP) uses molecular barcode (MBC) adapters and gene-specific primers (GSPs), enabling NGS-based immune chain mRNA interrogation from a single sample. This eliminates the need for opposing primers that bind within the highly variable V-segment, eliminating clonal dropout due to somatic mutation.

Aims: Our goal was to develop an NGS assay based on AMP that would enable IR characterization utilizing a minimal set of unidirectional GSPs and to reduce amplification bias through the use of MBC adapters.

Methods: Upon developing our AMP-based NGS assay, we validated its quantitative reproducibility and sensitivity. A peptide is isolated from PBMCs of healthy donors, B-cell chronic lymphocytic leukemia donors and formalin-fixed paraffin-embedded (FFPE) tissue.

Results: We developed the AMP-based NGS assays, Immunovar™ (IGH for B-cell and T-cell receptor) and CD8-Targeter sequencing, respectively. Both assays demonstrated high reproducibility between replicates with quantitative clone tracking down to 0.01%. The ability to determine isotype, clonotype and IGHV mutational status in a single assay was demonstrated. Preliminary TCR assay data indicates that CDR3 sequence capture is possible from FFPE tissue with clonotype calling being driven by input quantity, T-cell content, and, to a lesser degree, mRNA quality.

Summary/Conclusions: AMP-based NGS with MBC quantification and error-correction is a powerful method to characterize the immune repertoire.

**E1091**

SYNERGISTIC ANTITUMOR IMMUNITY BY DENDRITIC CELLS IN COMBINATION WITH POMALIDOMIDE AND DEXAMETHASONE IN A MURINE MYELOMA MODEL

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Background: Pomalidomide (Pom) plus dexamethasone (Dex) could be considered one of the new treatment options in patients with relapsed and/or refractory multiple myeloma (MM). Recently, several diverse agents would be combined to improve the therapeutic efficacy of immunotherapy.

Aims: In this study, we investigated the preclinical efficacy of combined therapy with dendritic cells (DCs) and Pom-Dex in a murine myeloma model.

Methods: After establishing myeloma-bearing mice, four treatment groups were designed to be a mimic protocol as like treatment in clinics as following: 1) PBS control, 2) DCs, 3) Pom + Dexam, and 4) DCs + Pom + Dexam. After vaccination, preclinical and in vivo immunological responses were evaluated.

Results: Among four treatment groups, DC combined with POM and DEXA strongly inhibited tumor growth, compared with other groups. In vitro immunological analyses revealed that these enhanced anti-tumor effects were closely associated with the decrease of regulatory cell populations, such as regulatory T cells (Treg) and type 2 macrophages (M2), and the increase of effector cell populations, including activated CD4 T cells, and type 1 macrophages (M1), accompanied with the activation of cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells in the splenocytes from vaccinated mice.

Conclusion: In this study, the DC combined with POM and DEXA synergistically enhance the anti-tumor immunity in a murine myeloma model, by skewing immune-suppressive status toward immune-suppressive status in tumor microenvironment.

**E1089**

DENDRITIC CELL VACCINATION COMBINED WITH LENALIDOMIDE AND PROGRAMMED DEATH-1 (PD-1) BLOCKADE HAS SYNERGISTICALLY INDUCED A MARKED TUMOR REGRESSION IN A MURINE MYELOMA MODEL

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Background: There is an emerging evidence that the maximal benefit of dendritic cell (DC)-based cancer immunotherapy may be achieved by combination with other therapies that act to immunomodulation and tumor microenvironment.

Aims: In this study, we tried to obtain the best efficacy of immunotherapy using DC vaccination in combination with lenalidomide and PD-1 blockade in a murine myeloma model.

Methods: After establishing myeloma-bearing mice, five treatment groups were designed to be a mimic protocol as like treatment in clinics as following: 1) PBS control, 2) DCs, 3) DCs + lenalidomide, 4) DCs + PD-1 blockade, and 5) DCs + lenalidomide + PD-1 blockade. After treatment, preclinical response and in vivo immunological responses were evaluated.

Results: DCs combined with lenalidomide and PD-1 blockade showed the best tumor regression among the study groups. These anti-tumor effects have meaningfully related to the decrease of immuno-regulatory populations, such as myeloid-derived suppressor cells (MDCs), M2 macrophages, and regulatory T cells (Treg) and the increase of effector immune cell populations, including CD4+ and CD8+ T cells, natural killer (NK) cells, and M1 macrophages, accompanied with the activation of cytotoxic T lymphocytes (CTLs) and NK cells in the splenocytes from the treated mice. Moreover, the level of immunosuppressive cytokines, such as TGF-β and IL-10, was significantly reduced in tumor microenvironment.

Summary/Conclusions: DC vaccination in combination with lenalidomide plus PD-1 blockade has synergistically induced a strong antitumor immunity by modulating tumor microenvironment in a murine myeloma model. This protocol will become a promising translational approach to improve the efficacy of immunotherapy in the field of MM.
E1092

ALTERATIONS IN T-CELL SUBPOPULATIONS AFTER CO-CULTURING WITH MSCS DERIVED FROM DIFFERENT DONORS

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Background: Study of interactions between lymphocytes and mesenchymal stromal cells (MSCs) in vitro revealed increase of HLA-DR expression on T-cells after co-cultivation with some MSCs samples. On lymphocytes derived from one donor the elevation of HLA-DR was observed after co-cultivation with half of MSCs samples (group A); on the others the HLA-DR expression level did not change (group B). MSCs were divided into two groups based on HLA-DR rise on lymphocytes. Study of T-cell subpopulations after interactions with MSCs could explain ineffectiveness of some MSCs as an immunomodulating agent in clinical applications.

Aims: The aim of the study was to discriminate variations in T-cell subpopulations, co-cultured with MSCs from two groups.

Methods: MSCs were isolated from bone marrow of 13 donors for allogeneic hematopoietic cells transplantation and cultured by a standard method. MSCs were seeded 10⁵ cells per flask, and then 10⁴ allogeneic lymphocytes from single donor were added to all MSCs cultures. For lymphocytes activation 5μg/ml phytohemagglutinin (PHA) was added to half of these cultures. Lymphocytes were removed from MSCs. Than MSCs were removed from the bottom of the flask by trypsin and expression of HLA-DR on their surface was measured by flow cytometry. Activation markers CD25, CD38, CD69, HLA-DR were studied by flow cytometry. CD45RA, PD-1 were studied by flow cytometry as well as distribution of naïve and effector T-cells were analyzed on 4th day of cultivation. p<0.05 was considered statistically significant; all data are presented as medium ± SEM.

Table 1.

Table. Subpopulations of lymphocytes (A) and MSCs (B) co-cultivated with group A and group B.

Results: Expression of HLA-DR on lymphocytes after 4 days of cultivation without MSCs did not change compared to 1st day. When lymphocytes were co-cultured with some MSCs samples expression of HLA-DR was higher. Elevated percentage of HLA-DR positive cells correlates between CD4+ and CD8+ cells (R²=0.932). Thus samples of MSCs were divided into two groups: in group A proportion of HLA-DR lymphocytes was 3 times greater than in group B. Subpopulations of lymphocytes co-cultured with MSCs from group A and B were compared. Subpopulations which significantly differed between groups A and B are presented in the table. In lymphocytes co-cultured with MSCs there were higher number of naïve cells compared to control (47.4±3.5% and 54.9±2.0% for group A and B respectively); naïve CD4+ cells were higher in group B, but number of TE was increased. Investigation of HLA-DR expression on MSCs after co-culturing with lymphocytes showed higher level of fluorescence signal (MFI) in group A then in group B (635±130 and 36.9±1.4% for lymphocytes cultured without MSCs, p=0.001). Group B showed lower number of EM and TM cells. Differences between groups were more pronounced when lymphocytes were activated. In group B proportion of HLA-DR CD4+ and CD8+ cells was significantly lower, compared to group A and control samples. At the same time the number of CM and PD-1+ CD4+ cells was lower in group B, but number of TE was increased. Investigation of HLA-DR expression on MSCs after co-culturing with lymphocytes showed higher level of fluorescence signal (MFI) in group A then in group B (635±130 vs 289±18, p=0.03). These data indicated that MSCs from group A had become more immunogenic after interaction with lymphocytes and could not show immunomodulating properties in same way as MSCs from group B.

Summary/Conclusions: The immunomodulatory properties of MSCs depend on the donor. This could explain why administration of MSCs is not always successful. Preliminary study of MSCs prior to their administration may be used to predict their efficiency in the future.

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E1093

GRANULOCYTE COLONY STIMULATING FACTOR AND ERYTHROPOIETIN ENTIRELY GIVEN FOR NEONATES RECOVERING FROM GUT SURGERIES: RANDOMIZED CONTROLLED TRIAL

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Background: Feeding intolerance is a common problem among neonates recovering from surgery for congenital abnormalities of the gastrointestinal tract (G.IT) such as small bowel atresia, omphalocele or gastrochisis. Feeding intolerance is a multifactorial process, but one of the important reasons is congenital maldevelopment of the small bowel villi. Disuse atrophy of the small bowel mucosa following several days of post-operative enteral feeding is one factor that can contribute to feeding intolerance. The human fetus swallows over 200 ml/kg/day of amniotic fluid and such swallowing is essential for normal small bowel development. Growth factors found in the amniotic fluid have been shown to promote proliferation of fetal intestinal cells. These growth factors include epidermal growth factor, granulocyte colony stimulating factor (G-CSF) and erythropoietin (EPO). We postulated that infants recovering from surgeries for congenital obstructive bowel abnormalities could be provided with physiologic quantities of recombinant human G-CSF and EPO by the intermittent orogastric or nasogastric administration of 20 mL/kg/day of sterile isotonic solution that would contain cytokine concentrations comparable to what they would have ingested from amniotic fluid in utero.

Aims: is to test a hypothesis suggesting that feeding tolerance could be improved in neonates recovering from surgeries for congenital obstructive bowel abnormalities by enterally administering recombinant human G-CSF and EPO included within similar isotonic solution administered over 200 mL/kg/day.

Methods: This double-blinded randomized controlled clinical trial was conducted on 40 neonates recovering from GIT surgeries for congenital bowel abnormalities. Hemodynamically unstable babies, and those with any contraindication to enteral feeding were excluded. Neonates were randomly divided postoperatively into 2 groups; 20 neonates received the test solution (called Simulated Amniotic Fluid-like solution given Enteraly; SAFE); 20 neonates enterally received distilled water (control). Treatment was started postoperative and the test solution (or distilled water) was discontinued when enteral intake reached 100cc/kg/day. Feeding tolerance and adverse effects of treatment (if any) were assessed.

Results: All the studied neonates tolerated the received solution well without side effects that could be attributed to its intake. The study group showed better feeding tolerance as reflected by earlier achievement of 50, 100, 120 and full enteral feeding with higher enteral caloric intake 7 days after Safe administration than control group. Improvement in neonatal outcomes and help to decrease morbidities from post-operative malnutrition and feeding intolerance. Enteral administration of rhG-CSF and rEPO may play a critical role in preventing violus atrophy, thereby, reducing feeding intolerance in neonates recovering from surgeries for congenital bowel abnormalities.

Summary/Conclusions: This study provides further insights on the improvement of neonatal outcomes and help to decrease morbidities from post-operative malnutrition and feeding intolerance. Enteral administration of rhG-CSF and rEPO may play a critical role in preventing violus atrophy, thereby, reducing feeding intolerance in neonates recovering from surgeries for congenital bowel abnormalities.

E1094

CORRECT PYRUVATE KINASE DEFICIENCY

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Background: Pyruvate Kinase Deficiency (PKD) is a rare erythroid metabolic disease caused by mutations in the PKLR gene which encodes the erythroid specific Pyruvate Kinase (RPK) enzyme. The defective enzyme fails to produce normal ATP levels and consequently, erythrocytes from PKD patients show an energetic imbalance and are susceptible to hemolysis. Site-specific hematopoietic stem cell gene therapy would be the safest approach to treat PKD patients. In this study, different gene editing approaches have been explored to correct PKD, either by the Knock-in of a PKLR cDNA sequence in the second intron or by the site-specific correction of specific mutations.

Aims: In the Knock-in system, that previously showed to correct the PKD phenotype of PKD-iPSC lines, a recombination matrix carrying codon optimized PKLR cDNA and a puromycin selection cassette was inserted in the second intron of the PKLR gene assisted by TALEN nucleases.

Methods: Thus, the therapeutic matrix together with specific TALENs as DNA plasmid or mRNA, for the second intron of PKLR were electroporated in purified CD34+ cells from healthy cord bloods. Cells were then expanded and puromycin selected to enrich the population for gene edited ones.
Results: Although a high toxicity and low efficiency were observed with the electroporation technique used, up to 96% colony forming units showed the specific integration. Experiments directed to improve efficacy and reduce toxicity were then conducted. A high percentage of gene edited HPCs were detected by shortening the cell expansion and puromycin selection periods. Importantly, gene edited HPCs were detected after infusion in immunodeficient (NSG) mice. Moreover, patient-specific correction has been developed aiming at the correction of PKD patient's specific mutations.

Summary/Conclusions: Overall, we showed that gene editing in engraftable HPCs is feasible, although the efficiency of the procedure should be further improved prior to consideration of these strategies in the clinic.

E1095
BLAST KINETICS AFTER NON-ENGRAFTING HAPLOIDENTICAL MICROTRANSPLANTATION IN PATIENTS WITH RFRAC TORY ACUTE MYELOID LEUKAEMIA
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Background: Multiple trials have showed that granulocyte colony-stimulating factor (G-CSF) -mobilized donor peripheral-blood stem cells (GPBSCs) based on allo-graft can be effective in mediating graft-versus-leukemia (GVL) effects and promote hematologic recovery without triggering of acute GVHD. Purpose: To analyze the efficacy of non-engraftment haploidentical cellular therapy for patients with refractory acute myeloid leukemia by assessment of bone marrow blast blast and hematopoietic cells percent kinetics. Methods: Seven patients (4 males 57.1%, 3 females 42.9%) with refractory acute myeloid leukemia were enrolled into this Phase I/II study. They were treated with chemotherapy including fludarabine 30mg/m², etoposide 100mg/m², endoxan 750mg/m² followed by infusion of haploidentical unmanipulated GPBSCs 24 hour after last chemotherapy infusion. Morphologic assessment of bone marrow blast kinetic by bone marrow aspiration conducted before therapy, D14 and D30 after therapy. Hematopoietic cells percent kinetics (Hematologic recovery) was assessed by Complete blood count every day till Day 40.

Results: At day +30, 6 patients were evaluable for response and one patient had died. One patients out of 7 showed PR, then developed CR after a second microtransplantation and the patient who died showed PR at D14 marrow evaluation (8% blast). So collectively objective response rate was 28.6%. The patient who developed CR was consolidated with an HLA-matched sibling transplant at day +75 from the 1st microtransplantation (day +50 from 2nd microtransplantation). Three patients attain neutrophil recovery with median time 29.7 days (range, 13-40 days), while the other five patient did not. Two patients attain platelet recovery with median time 34.5 days (range, 29-40 days), while the other five patient did not. The cellular therapy did not elicit statistically significant changes in bone marrow blast blast over time, F(2, 10)=1.558, p= .258, partial η²=.089, with bone marrow blast blast decreasing from pre-infusion blast blast (M=60%, SD=22.4%) to D14 blast blast (M=39.5%, SD=24.7%) then increased to D30 blast blast (M=54.8, SD=33.5%), in that order. Less than four previous chemotherapy, fludarabine-free previous chemotherapy and response naïve patients are the factors associated with good response to microtransplantation. There was a strong positive correlation between patient age (statistically significant), CR1 duration (statistically non-significant) and blast blast at D30, r= .842 and .693, p= .036 and p= .307 respectively. There was a moderate negative non-statistically significant correlation between CD34+ cell dose and blast blast at D30, r= -.498, p= .315.

Summary/Conclusions: The use of G-CSF primed hla-identical microtransplantation appears to be a biologically active therapy in patients with refractory AML, especially in patients received less than four previous chemotherapy, fludarabine-free previous chemotherapy, response naïve and young age patients.

E1096
ALTERATIONS IN T-CELL SUBPOPULATIONS AFTER CO-CULTIVATION WITH MULTIPOTENT MESENCHYMAL STEM CELLS
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Background: Lymphocyte population depends on immunological state of organism and varies in different diseases and during treatment. Multipotent mesenchymal stromal cells (MSCs) are widely used for cell therapy due to their immunomodulatory properties. Administration of MSCs is not only active, but also immunomodulatory properties of MSCs could be induced by different cytokines, e.g. IFN-γ. After injection MSCs interact with activated and non-activated lymphocytes. Changes in lymphocytes subpopulations characterize the influence of MSCs on immunological state.

Aims: The aim of the study was to determine the distribution of naive and effector cells in lymphocytes co-cultured with MSCs.

Methods: MSCs were derived from bone marrow of 13 donors (7 male and 6 female aged 22 to 62 years, median 27 years). MSCs were co-cultured with allo-geneic lymphocytes in a ratio of about 1:10 for 4 days and their basic properties were analyzed over time. Lymphocytes were activated by adding to the culture medium 5mg/ml of PHA (PHA-lymphocytes). Some MSCs were treated for 4 hours with 500 U/ml IFN-g (gMSCs). Activation markers CD25, CD38, CD69, HLA-DR and PD-1 were studied by flow cytometry as well as distribution of naive T-lymphocytes in CD45RA+CD62L+1 (2h), 2h and 4 days after co-cultivation with allogeneic lymphocytes. The proportion of CD4+ and CD8+ T-lymphocytes remained unchanged for 4 days. When co-cultured with MSCs gMSCs the proportion of CD45RA+CD62L+1 reduced by 30% (from 47.5±3.0% to 32.8±3.3%) in cultured lymphocytes. It did not happen in lymphocytes co-cultured with MSCs and gMSCs (p=0.001). At the same time in cultured lymphocytes to the fourth day the number of CD4+ effector memory cells increased in 1.8 times from 19.5±1.9% to 34.6±2.4%, which did not occur when co-cultured with both MSCs and gMSCs (p=0.001). Thus, co-culturing with MSCs or gMSCs prevented naive T-lymphocytes transition into effector cells. The proportion of CD4+PD-1+ cells increased from 8.2±1.1% to 10.9±0.7% by the 4th day of cultivation. When co-cultured with MSCs and gMSCs the proportion of PD-1+ cells increased by 8% (p=0.0125). The proportion of HLA-DR+ on both CD4+ and CD8+ cells lymphocytes remained unchanged for 4 days. When co-cultured with MSCs and gMSCs for 4 days there was a consistent increase in the proportion of CD4+/HLA-DR+ (8.1±1.17% to 15.6±1.1%, p=0.005) and CD8+/HLA-DR+ (from 9.7±0.8% to 26.0±3.7%, p=0.024) of allo-geneic lymphocytes. It did not happen in lymphocytes co-cultured with both MSCs and gMSCs (p=0.001).

Results: By the fourth day of incubation the proportion of naive CD4+ cells reduced from 30% (from 47.5±3.0% to 32.8±3.3%) in cultured lymphocytes. It did not happen in lymphocytes co-cultured with both MSCs and gMSCs (p=0.001). Thus, co-culturing with MSCs or gMSCs prevented naive T-lymphocytes transition into effector cells. The proportion of CD4+PD-1+ cells increased from 8.2±1.1% to 10.9±0.7% by the 4th day of cultivation. When co-cultured with MSCs and gMSCs the proportion of PD-1+ cells increased by 8% (p=0.0125). The proportion of HLA-DR+ on both CD4+ and CD8+ cells lymphocytes remained unchanged for 4 days. When co-cultured with MSCs and gMSCs for 4 days there was a consistent increase in the proportion of CD4+/HLA-DR+ (8.1±1.17% to 15.6±1.1%, p=0.005) and CD8+/HLA-DR+ (from 9.7±0.8% to 26.0±3.7%, p=0.024). So allo-geneic lymphocytes induced peptide presentation on lymphocytes. The proportion of CD4+ central memory cells increased in PHA-lymphocytes from 37.4±4.4 at 1st day to 68.2±6.5 at 4 th day. MSCs inhibited this increase - the proportion CD4+ central memory cells increased from 24.4±2.7% to 46.6±4.5% (p=0.047). Thus the interaction of PHA-lymphocytes with MSCs inhibited their activation and preserved naïve state.

Summary/Conclusions: The composition of lymphocyte population changes during cultivation. The proportion of naive cells reduced, while the number of effector cells and the proportion of PD-1+ increased, indicating lymphocyte activation probably due to the presence of xenogenic serum in the culture medium. Co-cultivation with MSCs maintained lymphocytes in not activated state. The interaction of activated lymphocytes with MSCs inhibits their activation and preserves naïve state. IFN-γ priming did not enhance MSCs inhibitory effect on lymphocyte activation. It is shown that MSCs both preserved naïve lymphocyte condition and have an inhibitory effect on their activation. The materials are supported by grant from the Russian Science Foundation, Project № 16-15-00102.
Aims: In order to develop a gene therapy clinical trial for PKD we are optimizing transduction protocols compatible with a clinical application.

Methods: Using a GMP-grade lentiviral vector production according to manufacturing processes of the GMP VIVEbioTECH (www.vivebiotech.com) using a solid phase bioreactor iCLELLis. These viral batches have been tested for transduction efficiency in healthy cryopreserved cord blood CD34+ cells compatible with different viral transduction and transduction efficiencies, compatible with a clinical application. Two cycles of transduction showed an increased level of transduction at limiting concentrations of the viral vector, improving the VCN up to 2-fold.

Summary/Conclusions: Transduction optimizations are being carried out in order to reduce the amount of viral vector needed to achieve optimal transduction efficiencies.

E1098

INTERACTION OF MULTIPOTENT MESENCHYMAL STROMAL CELLS WITH LYMPHOCYTES REDUCES THEIR IMMUNO PRIVILEGED PROPERTY

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Background: Multipotent mesenchymal stromal cells (MSCs) are widely used for cell therapy of autoimmune diseases and graft-versus-host disease. MSCs have long been reported to be hypoimmunogenic or ‘immune privileged’. The treatment of MSCs with interferon-g (IFNγ) increases their immunomodulating properties, but induce HLA-DR expression on their surface. When administered intravenously MSCs interact with activated and non-activated lymphocytes. It is impossible to follow the fate of MSCs in the recipient’s organism. The only way to study the changes in the properties of MSCs after intravenous administration is in vitro model.

Aims: The aim of the study was to investigate the properties of MSCs after interaction with lymphocytes.

Methods: MSCs were isolated from 13 bone marrow samples used for allo- geneic hematopoietic cells transplantation and cultured by a standard method. MSCs were seeded 10^5 cells per flask and a day later 500 units/mL of IFNγ were added for 4 hours to half of the cultures (gMSCs). Some cultures were seeded with 10^6 allogeneic lymphocytes, to half of these cultures 5mg/ml phytohemagglutinin (PHA) was added for lymphocytes activation (PHA-Lympho- cytes). For each of the MSCs cultures the mean fluorescent signal intensity level (MFI) of CD90 PE, CD54 APC, HLA-DR APC was measured. Relative expression level (REL) of CD10, CFH, PTGES, IL6, CSF1, ICAM-1 was analyzed in MSCs by RT-PCR. MFI and REL were investigated on the 1st, 2nd, 3rd and 4th days of cultivation.

Results: The elevated expression ICAM1 on manipulated MSCs may indicate an increase in their adhesive properties. IFNg treatment and interaction with lymphocytes induced HLA-DR expression significantly greater than IFNg licensing. IFNg increased the viability of MSCs when co-cultured with lymphocytes. Immunomodulating properties of MSCs were amplified both after IFNg priming and interaction with lymphocytes, so not dependent on IFNg source (exogenous or secreted by lymphocytes). The elevated expression ICAM1 on manipulated MSCs may indicate an increase in their adhesive properties. IFNg treatment and interaction with lymphocytes induced in MSCs the increase in relative expression level (REL) of factors involved in immunomodulation (IDO1, CFH, PTGES, IL6, CSF1).

Summary/Conclusions: We have defined the window of mammalian HE specification. The abrupt loss of ongoing HE recruitment at E10.25 suggests an early switch to the lymphoid fate.

E1099

SPECIFICATION OF MURINE HEMOGENIC ENDOTHELIAL HEMATOPOIETIC PRECURSORS CEASES ABRUPTLY BY E10.25 AND CONSTITUTES A FUNCTIONALLY HETEROGENEOUS POPULATION

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Background: Hematopoietic stem cells (HSCs) arise from hematopoietic endothelial (HE) precursors between embryonic day 10.5 (E10.5) and E12.5 of murine development, primarily in the aorta-gonad-mesonephros (AGM) region and the umbilical (UA) and vitelline arteries (VA). The window of specification of HE has not been defined in any mammalian system.

Aims: To define the precise window of specification of HE hematopoietic precursors and interrogate at the single cell level their functional heterogeneity.

Methods: Dams pregnant with Cdh5+ERT2-CreROSA26Confetti/+embryos were treated with tamoxifen (TAM) at E7.5, E8.5, E9.5, E10.5 or E11.5. Here, TAM induces the permanent and random labeling of endothelial cells and their progeny with one of the Confetti allele fluorescence reporters (YFP, GFP, CFP or RFP). The blood of resulting adult Cdh5+ERT2-CreROSA26Confetti/+ offspring was then examined for the presence of Confetti+ cells by flow cytometry. Clonal ex vivo assays: VE-Cadherin+CD45- cells, which contain HE, were isolated by FACS from E9.5, E10.5 or E11.5 embryos and co-cultured at limiting dilution either with OP9 stromal cells (OP9) or AGM-derived embryonal mesenchymal cells engineered to express Mury- AKT (AA-ECS), which both support hematopoietic output from HE ex vivo. Co-cultures were scored for hematopoietic colony formation, which were then analyzed for HE reconstituting activity and by flow cytometry for hematopoietic cell surface markers.

Results: To estimate the temporal window of TAM activity, CD45.2+ROSA26ERT2-CreConfetti+ bone marrow (BM) cells were transplanted into CD45.2+CD45.1+ recipients treated with TAM three, two, one or zero days before transplant. Only the PB of recipients treated on the same day of transplants was compatible, Confetti+ cells were found in the PB of recipients treated either OP9 stromal cells (OP9) or AGM-derived endothelial cells expressing the C-type lectin-like receptor 2 (CLEC2), which efficiently labels HE precursors with distinct functional output or the existence of a continuum of HE at different stages of maturation.

Summary/Conclusions: We have defined the window of mammalian HE specification. The abrupt loss of ongoing HE recruitment at E10.25 suggests an active mechanism that terminates this process. We also observed large phenotypic and functional variability amongst individual HE precursors examined throughout ontogeny.

E1110

C-TYPE LECTIN-LIKE RECEPTOR 2 SPECIFIES A FUNCTIONALLY DISTINCT SUBPOPULATION OF MEGAKARYOCYTE-BASED LONG-TERM HEMATOPOIETIC STEM CELLS

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Background: Recent studies have supported the model in which hematopoietic stem cell (HSC) compartment consists of functionally distinct subsets with discrete self-renewal and differentiation potentials. However, their immunophenotypes and the functional diversities remain poorly understood. We previously reported that the authentically identified HSC population includes a subset of cells expressing the C-type lectin-like receptor 2 (CLEC2), which give rise to megakaryocyte progenitors (MkPs) and megakaryocytes bypassing the pathway from common myeloid progenitor (CMP) to megakaryocyte/erythrocyte progenitor (MEP) (21th Congress of EHA, # P356, 2016).

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Hematopoiesis, stem cells and microenvironment

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Hematopoiesis, stem cells and microenvironment
E1101 PRE-TRANSPLANT DEFECTS OF BONE MARROW ENDOTHELIAL CELLS MAY CAUSE THE OCCURRENCE OF POOR GRAFT FUNCTION AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Poor graft function (PGF) is a serious complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT). PGF is defined as complete donor hemochromatosis with no residual or recurrent leukemia, but a hypo- or aplastic bone marrow (BM) with 2 or 3 of the following: (1) neutrophils ≤0.5×10^9/L; (2) platelets ≤20×10^9/L; and/or (3) hemoglobin concentration ≤70 g/L for at least 3 consecutive days after day +28 post-HSCT. The exact pathogenesis of PGF remains unclear. Mouse studies suggest that endothelial progenitor cells (EPCs), one of the major components in BM vascular microenvironment, modulate the proliferation, self-renewal and differentiation of hematopoietic progenitors (HSPCs), was examined in non-manipulated and BM-educated HSPCs, was examined in non-manipulated and BM-educated HSPCs. In this regard, we previously reported that CLEC2 expression on HSCs correlates with the lymphopoiesis of BM-educated HSPCs. Moreover, we recently reported that the impaired BM EPCs post-HSCT, which could be quantitatively and functionally improved by atorvastatin in vitro, may induce the occurrence of PGF (Bloom, 2016). However, whether the BM EPCs in subjects with PGF are impaired pre-HSCT and the reconstitution kinetics of BM EPCs post-HSCT are unknown. Here, we aimed to elucidate this question.

Aims: To investigate whether the BM EPCs in subjects with PGF are impaired pre-HSCT. To compare the reconstitution kinetics of BM EPCs, HSCs, and their ROS levels in subjects with PGF and good graft function (GFF) post-HSCT.

Methods: A total of 115 patients who will receive allo-HSCT were prospectively recruited and randomly selected as training group (n=32) and validation group (n=83). The percentage of BM CD45-CD34+VEGFR2+ EPCs, CD34+ HSCs, and reactive oxygen species (ROS) levels in EPCs and HSCs were evaluated in all of the enrolled patients pre-HSCT by flow cytometry. Furthermore, 59 patients were monitored for the frequency and ROS levels of BM EPCs and HSCs pre and post-HSCT by flow cytometry. In order to identify risk factors for PGF, pre-HSCT risk factors with a P<0.10 on univariate logistic analysis were included in the multivariate logistic regression analysis, and factors with a P<0.05 were considered independently associated with PGF.

Results: A total of 18 patients including 5 patients in training group (15.63%) and 13 subjects in validation group (15.67%) developed PGF post-HSCT. Both in training group and validation group pre-HSCT, significantly reduced percentage of BM EPCs were observed in PGF patients than those in GFF patients, whereas no significant differences were found in the percentage of BM HSCs between PGF and GFF patients. Furthermore, similar ROS levels were demonstrated in BM EPCs and HSCs between PGF and GFF patients. Although there was no difference in transplanted CD34+ cell dose between the PGF and GFF groups, significantly lower percentages of BM EPCs and HSCs, whereas remarkably higher ROS levels were observed in BM EPCs and HSCs in PGF groups than those in GFF groups at +1 month and +2 months post-HSCT. Moreover, inverse correlations were observed between BM EPCs frequency and their ROS levels post-HSCT, as well as BM HSC frequency and their ROS levels post-HSCT. Multivariate analyses revealed that the reduced BM EPCs and the disease status pre-HSCT were independent risk factors for the occurrence of PGF following allo-HSCT.

Summary/Conclusions: We identified that patients with impaired BM EPCs pre-transplant were at a high risk for the occurrence of PGF post-allo-transplant. More consistent low percentage and high level ROS of BM progenitor cells in PGF patients than those in GFF patients and the disease status pre HSCT were independent risk factors for the occurrence of PGF following allo-HSCT.
Background: GATA4 is a transcription factor expressed in mesoderm and endoderm during development. Members of the family such as GATA1-3, but not GATA4, are critically involved in hematopoiesis. An enhancer (G2) of the mouse GATA4 gene directs its expression throughout the lateral mesoderm and the allantois, beginning at E7.5, becoming restricted to the septum transversum by E10.5, and disappearing by midgestation (Rojas et al., Development, 2005, 132:3405). Our previous work has shown that inactivation of Gata4 using this G2-Cre driver is lethal by midgestation (Delgado et al., Hepatology, 2014, 59:2358). The anemia observed in the G2-Cre;Gata4floxed/floxed embryos was attributed to a failure in the expansion of the hematopoietic progenitors in the fetal liver. Interestingly, a small population of hepatic YFP+ cells was positive for leukocyte and megakaryocyte markers, suggesting that a lineage of hematopoietic cells could derive from GATA4 expressing progenitors.

Aims: To study in our murine models the origin and properties of the hematopoietic lineage derived from progenitors expressing GATA4 under control of the G2 enhancer.

Methods: We analyzed hematopoietic organs of G2-Gata4Cre;R26R YFP mice, adults and embryos, by flow cytometry, RT-PCR and confocal microscopy. Cells obtained from different tissues were cultured and transplanted to analyze in vitro and in vivo potential.

Results: YFP+ cells represented about 20% of the hematopoietic system of adult mice and contributed in the same proportion to the lymphoid, myeloid and erythroid lineages. Adult YFP+ hematopoietic stem cells (Figure 1) constituted a long-term repopulating, transplantable population. Fetal YFP+ hematopoietic progenitors were much more abundant in the placenta than in the fetal liver. These placental YFP+ progenitors were clonogenic in the MethoCult assay and fully reconstituted hematopoiesis in myeloablated mice (Caféte et al., Haematologica. doi: 10.3324/haematol.2016.155812. [Epub ahead of print]).

Summary/Conclusions: A lineage of adult hematopoietic stem cells in mice is characterized by the expression of GATA4 in their embryonic progenitors and probably by its embryonic (placental) origin. Both lineages basically showed similar physiological behavior in normal mice, but this finding raises a number of questions, for example: Does this hematopoietic stem cell subpopulation show a different response in pathophysiological conditions? Does this subpopulation show a different profile of gene expression? Does a similar heterogeneity exist in human HSCs? We are currently investigating the transcriptome of the G2-GATA4 lineage HSC in order to answer these questions.
Methods: K562 (BCR-ABL positive chronic myeloid leukemia in blast crisis)-Luciferase-control or K562-Luciferase-SZF1/2NZF89 cells were directly injected into the femurs of NSG mice and tumor development was monitored by bioluminescence. Furthermore, K562 cells with or without SZF1/2NZF89 overexpression were studied by proliferation assay, cytometry, flow cytometry, cell cycle analysis, cyclin B1 expression and beta-galactosidase assay. Results: K562-dependent tumor growth was efficiently inhibited in NSG mice transplanted with K562-Luc-control-cells, leading to significantly prolonged survival, demonstrating a strong tumor suppressive potential of SZF1/2NZF89 in vivo. In vitro, overexpression of SZF1/2NZF89 dramatically inhibited proliferation of K562 cells, which, instead of dying, became giant and dysplastic, without other significant morphological changes and in absence of polyloidcy. Cell cycle analysis revealed a blockade in G2/M phase, with cyclin B1 accumulation characteristic for mitotic arrest. As suggested by morphology and beta-galactosidase assay, cell cycle arrest was leading to premature senescence. Summary/Conclusions: SZF1/2NZF89 controls survival of hematopoietic cells mediated by mitotic arrest and premature senescence, exhibiting tumor suppressive functions in vivo.

E1109
THE FUNCTIONAL RELEVANCE OF DNMT3A SPLICE VARIANTS IN HEMATOPOIETIC DIFFERENTIATION
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Background: DNA methyltransferase 3A (DNMT3A) plays a pivotal role for de novo DNA methylation (DNAm) during development. It seems to be of particular relevance in hematopoietic differentiation because it is frequently mutated in acute myeloid leukemia or clonal hematopoiesis. So far, it is unclear how DNMT3A governs gene expression. Here, we performed a screening to identify lineage-specific DNAm patterns, it is conceivable that this can at least partly be attributed to alternative splicing of DNMT3A.
Aims: In this study, we followed the hypothesis that specific splice variants of DNMT3A impact on hematopoietic differentiation or DNAm patterns. Therefore we addressed the role of specific splice variants of DNMT3A in hematopoietic stem and progenitor cells (HSPCs).
Methods: Expression of DNMT3A splice variants was modulated in HSPCs: transcript 1+3 (Tr.1+3), transcript 2 (Tr.2), or transcript 4 (Tr.4) of DNMT3A were either knocked down by short hairpin RNA or constitutively overexpressed by lentiviral infection. Expression changes were validated by qRT-PCR. Subsequently, we evaluated the impact on colony formation potential (CFU assay), proliferation (CFSE assay), and the immunophenotype (CD34+ and CD133+). Global DNAm profiles were generated with the Infinium HumanMethylation450 BeadChip platform and gene expression profiles with the Human Affymetrix Gene ST1.0 platform.
Results: Downregulation of either Tr.2 or Tr.4 reduced the proliferation rate of HSPCs significantly (n=3, p<0.05). HSPCs maintained CD34 expression for a higher number of cell divisions upon knockdown of Tr.2 (n=3, p<0.05). In colony forming unit (CFU) assays downregulation of Tr.4 resulted in a clear bias towards erythroid colonies (n=3, p<0.05). Overall, CFU frequency was reduced by knockdown of DNMT3A transcripts, whereas it was increased by overexpression. Subsequently, we analyzed the impact of specific DNMT3A variants on the DNAm patterns: several CpG sites revealed significant differences in DNAm levels upon knockdown of Tr.2 and 0.48%±0.13 osteoclasts’ area, respectively. Moreover, among the Pro-B cells, only those expressing M-CSF receptor (CD115) could transdifferentiate into osteoclasts (16%±3.7 vs. 0.79%±0.28 and 0.48%±0.13 osteoclasts’ area, respectively).
Aims: We set to determine whether B cells can transdifferentiate to osteoclasts and to assess the effect of EPO on this process.
Methods: Experiments were conducted on C57BL/6j or CD19-Cre;R26R-EYFP, 8-12-week-old female mice in accordance and with the approval of the Institutional Animal Care and Use Committee of Tel-Aviv University (M-14-043). BM cells were flushed from femurs, tibiae, and pelvic bone and red blood cells were lysed. Cells were stained with labelled anti-mouse antibodies: PE-B220, FITC-CD19, PerCP-igM, PeCy7-CD43, and APC-M-CSF receptor/CD115; and sorted by flow cytometry. Cells were then cultured in α-MEM containing 10% fetal bovine serum, M-CSF and RANKL. Multinucleated osteoclasts were stained for tartrate-resistant acid phosphatase (TRAP) and pit resorption was assessed.

Figure 1: Osteoclastogenesis in vitro from sorted B cells. (A) Transdifferentiation of 150,000 cells/well CD19-Cre;R26R-EYFP into osteoclasts. (B) TRAP staining. Confocal images (x20 magnification) (C) TRAP staining of osteoclasts derived from the indicated sorted cells from BM (10,000 cells/well) and cultured with M-CSF and RANKL. Left – Pro-B cells expressing CD115 (B220+CD19+CD43+igM+GM+), not Pre-B (B220+CD19+CD43+igM+GM+) or immature B cells (B220+CD19+CD43+igM+GM+). Right – Pro-B cells not expressing CD115 (B220+CD19+CD43+igM+GM+CD115). Data are means±SEM of osteoclast area, n=5 mice in each group; *p<0.05. (D) Pit resorption area from the indicated sorted cells cultured on calcium phosphate-coated plates. (D) TRAP staining of osteoclasts derived from sorted Pro-B cells (B220+CD19+CD43+igM+GM+CD115). Data are means±SEM of osteoclast area, n=5 mice in each group; *p<0.05. (E) Pit resorption area from the indicated sorted cells cultured on calcium phosphate-coated plates. (D) TRAP staining of osteoclasts derived from sorted Pro-B cells (B220+CD19+CD43+igM+GM+CD115). Data are means±SEM of osteoclast area, n=5 mice in each group; *p<0.05. (F) Pit resorption area from the indicated sorted cells cultured on calcium phosphate-coated plates. (D) TRAP staining of osteoclasts derived from sorted Pro-B cells (B220+CD19+CD43+igM+GM+CD115). Data are means±SEM of osteoclast area, n=5 mice in each group; *p<0.05. (G) Pit resorption area from the indicated sorted cells cultured on calcium phosphate-coated plates. (D) TRAP staining of osteoclasts derived from sorted Pro-B cells (B220+CD19+CD43+igM+GM+CD115). Data are means±SEM of osteoclast area, n=5 mice in each group; *p<0.05. Summary/Conclusions: Taken together, our data suggest a new physio-pathological role for BM B-cell precursors in bone metabolism via their capacity to differentiate into functional osteoclasts, and a possible role for EPO in this process.
Bone marrow myelopoiesis independently of canonical Notch signaling
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Background: Notch signaling is a highly conserved pathway important in multiple developmental processes. Canonical signaling through all Notch receptors converges on the Csl transcription factor recombination signal binding protein for immunoglobulin kappa J region (Rbpj). In haematopoiesis, Notch is critical for the development of the hematopoietic stem cells (HSCs) in the embryo and in thymic T cell development. Contrastingly, canonical Notch signaling has been shown to be dispensable for HSC homeostasis in the adult bone marrow (aBM). Recent studies have however suggested a role of Notch in promoting megakaryocyte (Mk) and erythrocyte (E) development as well as in suppressing granulocyte-macrophage (GM) progenitor expansion and acting as a tumor-suppressor in myeloid malignancies. However, these findings were largely made through genetic approaches potentially also affecting regulatory mechanisms. We next investigated whether loss of canonical Notch signaling might be applicable to relieve the emergence of definitive hematopoietic stem cells (HSCs) in the adult marrow as well as after BM transplantation.

Aims: To unambiguously investigate the role of canonical Notch signaling in aBM myelopoiesis, in steady-state and following transplantation.

Methods: BS-SLJDCD4.5, Rbpjfl/fl, Mx1-Cre, Vav-Cre and Vwf-eGFP BAC mice were used. Flow cytometry (FACS) was applied for phenotypic analyses.

Results: FACS staging of GM, Mk and E progenitors in aBM of lox-flanked Rbpj mice crossed to both Mx1-Cre and the pan-haematopoietic Vav-Cre strains was applied. As expected, HSCs were unaffected. Not previously investigated, FACS allows a direct comparison of GM, Mk and E progenitors with Rbpj-deficient defects, at any progenitor stage, in Rbpj-deficient mice. To demonstrate that this lack of a phenotype was not due to BM cells escaping Rbpj deletion, we FACS purified HSCs and all GM, Mk and E progenitor stages from Rbpj-deficient mice and verified a virtually complete depletion of Rbpj in all populations. In further agreement with canonical Notch signaling not being required for steady-state generation, maintenance or stepwise differentiation of adult GM, Mk and E progenitors, the number of GM, Mk and E colonies generated from unfractionated aBM cells as well as circulating platelet counts were also unaffected.

Conclusions: We next sought to address whether loss of Notch signaling would uncover a role of the Notch pathway in regulation of GM, Mk and E progenitors by establishing BM chimeras in which Rbpj-deficient progenitors compete with wild type (WT) progenitors for replenishment and differentiation in lethally irradiated recipients. No deficiencies were observed in the replenishment of HSCs, and any stages of GM, Mk and E progenitors in mice competitively transplanted with Rbpj-deficient as compared to control WT BM cells. Moreover, transplanted Rbpj-deficient and control progenitors contributed equally well to platelet reconstitution. We next investigated whether loss of canonical Notch signaling might nevertheless impact on expression of genes for key regulators the Mk and E lineages at distinct progenitor stages for these lineages, as previously implicated. Notably, transcript levels of genes encoding key Mk/E regulators were unaffected in Rbpj-deficient Mk/E progenitors. In previous studies, expression of Notch target genes in Mk and E progenitors in aBM has been implicated as reflecting activation through Notch signaling. However, since the expression of Notch targets might also be regulated by other pathways besides Notch pathway, we investigated whether the expression of key Notch target genes (Hes1, Hes5, Notch1, and Jag1) in Mk/E progenitors in aBM-dependent on canonical Notch signaling. Neither in HSCs or any Mk/E progenitor was the expression of these Notch genes negatively affected by Rbpj-deficiency, demonstrating that their low expression levels in aBM HSCs and Mk/E is independent of canonical Notch signaling.

Summary/Conclusions: Studies implicating canonical Notch signaling as a critical regulator of aBM Mk, E and G progenitors potentially failed to target only canonical Notch signaling. Herein, we demonstrate that canonical Notch signaling is dispensable for generation and replenishment of Mk, E and GM progenitors in aBM in steady-state as well as following BM transplantation.

Identification of novel human hematopoietic stem cell subpopulations via comprehensive surface marker analysis
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Background: All hematopoietic cells are derived from hematopoietic stem cells (HSCs), which exhibit capacities for multilineage differentiation and long-term self-renewal. Human HSCs can be isolated by Fluorescence-activated cell sorting (FACS) with the combination of several surface markers, such as CD34+, CD45RA-, and CD38- or CD45RO, as LSC. The presence of functionally heterogeneous subpopulations, including multi-potent and/or lineage- biased progenitors (Notta:2016hh) and HSC-like populations with reduced self-renewal capacity (Notta:2011bg); however, prospective isolation of bona fide human HSCs is still challenging due, at least in part, to the lack of specific functional assays.

Aims: The goal of this study is to identify a novel HSC-specific surface marker(s) that enables prospective isolation of functionally-distant HSC subpopulations.

Methods: We examined expression levels of 342 cell surface markers in the HSCs (Lin-CD34+CD38-CD45RA-CD90+) by FACS using commercially-available antibodies. Single-cell gene expression profiling of isolated subfractions were performed using Fluidigm C1 system in combination with BioMark. Differentiation potential of each HSC fraction was assessed by single-cell colony assays in methylcellulose. In vitro lineage tracing in liquid culture were performed to determine hierarchical relationships among subfractions.

Results: Among 342 cell surface proteins examined, only CD35, CD115 and CD212 were detected in the HSC fraction. We focused on CD35, which is also known as complement receptor type 1 (CR1), as its expression was most distinct among the three markers. CD35-positive population accounted for the emergence of the human HSCs, defined as Lin-CD34+CD38-CD45RA-CD90+ cells, in adult bone marrow and cord blood. HSCs exhibited multi-lineage reconstitution capacity without lineage-biased differentiation in a single-cell colony assay regardless of the CD35 levels. CD35+HSCs gave rise to CD35- HSCs in lineage tracing experiments, suggesting that CD35+ HSCs reside upstream of CD35- HSCs in the hematopoietic differentiation. Single-cell gene expression profiling of CD35-positive or -negative HSCs indicated that CD35+HSCs, but not CD35- HSCs, are phenotypically homogenous, expressing cell cycle-related genes and lineage-specific markers at low levels.

Summary/Conclusions: Our data suggest that HSCs can be further subdivided into subfractions based on CD35 levels. CD35 might be a useful marker to prospectively isolate the most primitive human HSC fraction. In vivo functional assays using xenotransplantation models are currently underway, and the results will be discussed at the meeting.
fibres. Treatment of K562 or HL60 cells with imatinib or doxorubicin respectively resulted in a lower level of apoptosis in cells grown on the 3D scaffold compared to those grown in 2D culture. Further development of this 3D culture by adding stromal HS-S to the scaffold reduced even further the sensitivity of K562 or HL60 to imatinib or doxorubicin, respectively.

**Figure 1.**

**Summary/Conclusions:** The relative resistance to either imatinib or doxorubicin that we observed in cells grown in 3D culture supports a role for the bone marrow matrix in the protection of leukemic cells against chemotherapeutic agents. A combination of the PMMA-HA with HS-5 cells made this system more similar to the bone marrow microenvironment as this is a model in which all the basic components of the bone marrow microenvironment such as scaffold, stromal cells and cytokines (secreted by HS-5) are present. The results of this study show adding extra complexity to the microenvironment changes the sensitivity of the cells to therapeutic agents, better recapitulating the situation observed in-vitro. Three dimensional cultures using the PMMA-HA/HS-5 model may prove useful in the investigation of therapy resistance in leukemia and for the discovery of new agents capable of eradicating quiescent leukemic stem cells.

**E1113**

**WHOLE EXOME SEQUENCING REVEALED SEQUENTIAL GAIN OF MUTATIONS IN TWO CASES OF DONOR CELL HAEATOMATOPOIETIC TRANSPLANTATION**

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**Background:** The leukemic transformation of otherwise healthy donor stem cells provides a useful in vivo model to study the mechanisms involved in leukemogenesis.

**Aims:** We report two cases of donor-cell derived haematological malignancy in which whole-exome sequencing (WES) was performed in bone marrow (BM) samples from recipient at different times after allogeneic haematopoietic stem cell transplantation (allo-HSCT) in order to study the dynamics of emergence of mutations that precede the development of donor cell leukemia (DCL) and donor cell myelodysplastic syndrome (DC-MDS).

**E1114**

**LEUKEMIC STEM CELL-RELATED mRNA EXPRESSION ANALYSIS USING A NOVEL FLOW CYTOMETRY-BASED ASSAY**

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**Background:** Gene expression analysis of protein-coding (mRNA) and non-coding RNA in paediatric and adult acute myeloid leukaemia (AML) has become of paramount importance for therapeutic decision-making, revealing prognostic information and for the identification of novel therapeutic targets. AML is a clinically, phenotypically and molecularly heterogeneous haematological malignancy, with different leukemic cell populations organized in a hierarchical fashion, and leukemic stem cells (LSCs) residing at the apex herein. Unfortunately, gene expression profiling is commonly performed on unfractonated bulk samples, leading to “expression averaging” of these heterogeneous cell populations. Multicolor flow cytometry (FCM) is capable of distinguishing heterogeneous cell populations based on the phenotypic characterization at a single-cell level. However, fluorochrome-conjugated antibodies are not available for intracellular RNA targets.

**Aims:** To evaluate the applicability of a novel flow cytometry-based technique, PrimeFlow™ RNA assay, to measure cell-of-interest RNA expressions in heterogeneous AML samples.

**Methods:** Technical assessment was performed using six neuroblastoma cell lines with varying levels of MYCN gene amplification. Correlation to expression data obtained by the gold standard RT-qPCR, performance in rare (0.1%) cell populations, effects of cryopreservation and off-target effects were evaluated. Next, diagnostic material of de novo AML patients was used to measure target gene (Wilms tumor 1 (WT1)) and reference gene (RPL13a, GAPD) expression. Expression analysis was performed in unfractonated bulk leukemic cells as well as blasts and rare subsets of leukemic cells, e.g. LSCs. FCM analyses were performed on a FACScanIn II (BD Biosciences) with set-up according to EuroFlow guidelines. Infinicyt™ (Cyognos®) was used for data analysis and mean fluorescence intensities (MFI) values (with/without normalisation) were interpreted. P-values < 0.05 were considered significant.

**Results:** mRNA expression quantified by PrimeFlow™ significantly correlated with data obtained by RT-qPCR and remained detectable in rare (0.1%) cell populations. WT1 expression was shown to be statistically significantly higher in bulk leukemic cells of those patients characterized by WT1 overexpression, as defined by RT-qPCR, showing a mean 52% MFI upregulation by PrimeFlow. ARHGAP26 and MCM2 expression was shown to be statistically significantly higher in CD34+CD38− cells and to detect new mutations involved in the emergence of AML.

**Figure 1.**

**Methods:** Case 1: A 43-year-old female diagnosed with lymphoblastic leukemia-B in 1991, who developed acute myeloid leukemia (AML) with normal karyotype, NPM1+ of donor origin 16 months after unrelated cord blood transplantation (UCBT). Case 2: A 65-year-old male diagnosed with mantle cell lymphoma, who developed MDS 45.XX,-7.del(12)(p12) of donor origin, 57 months after allo-geneic BM transplant from his HLA-identical brother. WES (SureSelect-XT Human-exon 50Mb) was performed by next generation sequencing (HiSeq) on donor stem cells (SCs) infused as well as on BM samples from recipient after allo-HSCT. The exome of donor SCs and 5 BM samples, from case 1, were aligned to the human reference genome (GRCh 37/hg19) and donor SCs and 9 BM samples were aligned to GRCh 38/hg38 in the second case.

**Results:** WES analysis revealed progressive emergence of multiple somatic mutations probably related to the development of leukaemia in bone marrow samples post allo-HSCT (Figure 1). Both SCs showed alterations that may be involved in leukemogenesis. (Case 1: SH2B3 and case 2: KMT2C, KMT2A, ARHGAP26 and monosomy 7). Somatic mutations, acquired over time, fall into genes that play well-established roles in signalling pathways. Mutations in leukemic subclones that disappear after chemotherapy were indentified, as well as the acquisition of new mutations in resistant subclones. We propose a possible model of leukemogenesis in these cases (Figure 2).

**Summary/Conclusions:** The present study reveals a process of sequential clinical transitions, promoted by the acquisition of additional somatic mutations in donor hematopoietic cells. Detection of inheritable or acquired gene mutations in donor associated with predisposition to haematological malignancies could have clinical implications for the patients undergoing to allo-HSCT. Although the cause of donor cell derived haematological malignancy onset seems to be multifactorial, the infuxion of a SCU with pre-leukemic potential in a context of multifactorial, the infusion of a SCU with pre-leukemic potential in a context of...
Summary/Conclusions: Key mRNA target expressions in AML, e.g. WT1 gene expression, could be evaluated using PrimeFlow™ RNA assay, including rare and heterogeneous cell populations herein, e.g. LSCs. This study demonstrates that PrimeFlow™ is a technique of interest for the discovery of novel LSC-specific targets.

E1115

POTENTIAL PREDISPOSING GERMLINE MUTATIONS IN PATIENTS WITH CONCOMITANT MYELOID AND LYMPHOID MALIGNANCIES

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Background: Recent findings have suggested that mutations predisposing the development of either acute myeloid leukemia (AML) or chronic lymphocytic leukemia (CLL) may arise in pre-leukemic hematological stem cells. In addition, genes involved in epigenetic regulation, such as TET2, and RNA processing, such as SF3B1, are mutated in both myeloid and lymphoid malignancies. This could indicate a possible genetic link between myeloid and lymphoid malignancies. Therapy related AML (t-AML) is a known complication to treatment with cytotoxic drugs such as alkylating agents and topoisomerase inhibitors. The susceptibility of developing t-AML has been associated with variation in DNA-repair pathways, drug metabolism and transport.

Aims: In this study, we aimed to investigate a possible common genetic origin of hematological cancers in patients with concomitant CLL and de novo AML or myelodysplastic syndrome (MDS) and in patients with concomitant therapy-related AML (t-AML) and CLL.

Methods: The presence of concomitant lymphoid and myeloid malignancies in patients is rare, however we managed to include 3 patients with de novo AML and CLL, one patient with MDS and CLL, one patient with chronic myelomonocytic leukemia (CMML) and CLL, and two patients with t-AML and CLL. The patients' diagnoses were based on the evaluation of the morphology, immunohistochemistry, cytogenetics, and flow cytometry analysis in accordance to the WHO classification. For each patient mononuclear cells (MNCs) from blood or bone marrow were isolated using density centrifugation and used for fluorescence activated cell sorting (FACS) of the malignant clones and the T-cells. Paired end exome sequencing (2x150) aiming for an average coverage of 50-100x was performed using either the HiSeq2500 or NextSeq500 platforms from Illumina. Raw sequencing data was processed using CASAVA-1.8.2. Mapping to the human genome (hg19/GRCh37 UCSC) was performed using the CLC Biomedical Genomics Workbench (Qiagen) m clonal cell software. Variants with a frequency of 5% or above were called.

Results: We identified possible pre-disposing germline mutations in all 7 patients by comparing variants between the myeloid malignant clone, CLL cells, and T cells, as well as using saliva to aid in characterizing the mutations as either germline or only present in the hematological compartment. In all the patients except one with de novo AML and CLL, we identified a potential damaging germline variant in a DNA-repair related gene, such as ATM (387dupA, D130fs*4), SMARCAL1 (2114C>T, T705I), HELQ (393_397delAGGTG, 1213V*16), SWI5 (585C>T, R218*), LG1(2186A>G, Q761R) and PRKDC(802G>A, C301Y). In the remaining patient with concomitant de novo AML and CLL, we identified a potential damaging germline variant in an epigenetic regulator believed to play a role in normal and malignant hematopoiesis, KDM2B(44delC, P15fs*92). Furthermore, we identified the somatic mutational landscapes of the malignant clones using T-cells as germline tissue for the evaluation of the mutational landscapes of the malignant clones in patients except one with de novo AML and CLL. We identified a potential damaging germline variant in CHIP and AML in the DNMT3A gene by several different studies. Figure 1 clearly illustrates the mutations in comparison to AML and in CHIP. Only 13% of all reported mutations were found at the R882 residue in CHIP, while in AML 60% DNMT3A mutations were found at the R882H mutations.

Summary/Conclusions: Analysis of the mutational landscape of CHIP has clearly highlighted the role of DNMT3A mutations in concomitant therapy-related AML in older healthy individuals, the significance of such preleukaemic clones is yet to be determined.

E1117

NEXT-GENERATION REFERENCE INTERVALS FOR PEDIATRIC HEMATOLOGY

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Background: Interpretation of hematology analytes in children is challenging due to extensive changes in hematopoiesis with age leading to pronounced sex- and age-specific dynamics. To facilitate clinical decision making based on quantitative hematological test results, reference intervals are used to classify samples according to upper and lower limits, and age-related change is represented using reference intervals partitioned into separate age groups. However, this approach can only approximate the continuous physiological dynamics of hematological analytes in childhood and does not enable appropriate quantification of test results in relation to the reference distribution. Conversely, percentile charts as used in anthropometric quantities (e.g. pediatric weight-for-age charts) would allow adequate appreciation of pediatric hematological test results. However, the ethical and practical challenges unique to pediatric reference intervals have restricted the creation of such percentile charts, and limitations in current approaches to laboratory test result display prevent their integration into clinical decision making.

Aims: To create percentile charts for hematological analytes from birth to adulthood using a data-mining approach and to demonstrate their integration into clinical care with benefits in clinical decision making.
Methods: We applied a data-mining algorithm to generate percentile charts for hematology analytes using laboratory data collected during the clinical care of patients. A total of 9,517,245 samples from 343,463 patients (72,614–337,011 samples per analyte) from 8 German tertiary care centers and 2 German laboratory service providers were examined. Percentile charts were calculated using an established statistical approach which extracts the proportion of samples from healthy individuals from the unfiltered input dataset containing both non-pathologic and pathologic samples. To evaluate the clinical benefit of hematology test result interpretation using percentile charts, accuracy and speed of pediatricians assessing eight different predefined clinical situations were measured in comparison to conventional test result representations.

Results: We created percentile charts for hematology analytes in girls and boys from birth to 18 years which can be used as common reference intervals. Results are provided for hemoglobin, hematocrit, red cell indices, red cell count, red cell distribution width, white cell count, and platelet count, example charts for hemoglobin, mean corpuscular volume, and platelet count are shown in the accompanying figure. A web application at www.pedref.org/hematology demonstrates hematology test result interpretation using percentile charts and z-scores with special consideration of pediatric dynamics. Comparison of pediatricians’ decision times when assessing different clinical scenarios using percentile charts and conventional representations shows more correct decisions (75.9% vs 68.4%, p<0.01) which are made in shorter time (2.7 s vs 3.8 s, p<0.01) when using percentile charts.

Summary/Conclusions: The created percentile charts enable the appropriate differential diagnosis of changes in hematology analytes due to disease and changes due to physiological development. Integration of suitable forms of result reporting using the provided percentile charts into clinical decision making improves assessment of the unique dynamics in pediatric hematology.

E1118

GROWTH FACTOR INDEPENDENCE 1 (GFI1) REGULATES THE AML SUPPORTING FUNCTION OF MESENCHYMAL STROMAL CELLS

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Background: Mesenchymal stromal cells (MSCs) harbor and support the function of normal hematopoietic stem cells. Less is known about their interaction with leukemic cells, e.g. in acute myeloid leukemia (AML). The prognosis of AML, a clonal malignant disease of the bone marrow (BM), is still poor with only 25% of patients living longer than 5 years.

Aims: In the current study, we investigated the interaction between MSCs and AML cells, and we also investigated the underlying molecular mechanism.

Methods: We used cell cultures using primary cells from human and mice and cell lines of MSCs and AML cells. Different Mouse models of human AML were used in our study to confirm the results obtained from human sample. MSCs were characterized by differentiation assay, flow cytometry and RT-PCR. Matrigel test was also applied in this study.

Results: MSCs from AML patients called AML-associated MSCs (AMSCs) or from murine models of human leukemia enhance significantly in vitro the growth of leukemic cells compared to AML cells growing without MSCs or in presence of MSCs from non-leukemic patients or mice. Among other, AMSCs increased entry of leukemic cells into the cell cycle, and at the same time protected the leukemia cells against exogenous toxic events such as chemotherapy or irradiation. The interaction between AMSCs and leukemia cells is dependent on cell-to-cell contact. In vivo, absolute and relative numbers of AMSCs and other stromal cells, i.e. endothelial cells and osteoblast lineage cells were highly expanded in the BM of mice modeling of human AML. AMSCs showed a higher efficiency of capillary tube formation in the matrigel assay than normal MSCs which gives an additional indication that AMSCs were polarized by leukemia cells towards a tumor-supporting state. On a molecular level, the polarization of MSCs towards an AML-supporting state depends on upregulated expression of the transcription factor Growth factor independence 1 (Gfi1). Loss of Gfi1 diminished the tumor-supporting state of AML-associated MSCs.

Summary/Conclusions: We conclude that leukemia cells polarize AMSCs towards a leukemia-supporting state in a Gfi1-dependent manner, which could open the way to new therapeutic approaches.
BASELINE LEUKOCYTE AND EOSINOPHIL COUNTS PREDICT OUTCOME IN RELAPSED OR REFRactory CLASSICAL Hodgkin lymphoma PATIENTs TREATED WITH PD1 INHIBITION

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Background: Despite encouraging efficacy of anti-PD1 antibodies in relapsed or refractory (r/r) classical Hodgkin lymphoma (cHL), not all patients achieve a lasting response, with few complete remissions (CR) observed. Thus, identification of predictive biomarkers is important. Recently, two models using readily available differential blood count parameters have been suggested to predict outcome in melanoma patients treated with immune checkpoint inhibition.

Aims: In this study, we aimed to identify baseline differential blood count parameters associated with response and progression-free survival (PFS) in r/r cHL patients treated with anti-PD1 antibody nivolumab.

Methods: We retrospectively investigated baseline differential blood count parameters and their association with response and progression-free survival (PFS) in 30 r/r cHL patients treated with the anti-PD1 antibody nivolumab. All 30 patients had previously received multiple lines of treatment, including treatment with high dose chemotherapy followed by autologous stem cell transplant (ASCT) for r/r disease; the median number of prior treatment lines was 5 (2-11) and 21 patients received prior brentuximab vedotin. To investigate the association of baseline blood count parameters (white blood cell count (WBC), relative monocyte count (RMC), relative neutrophil count (RNC), relative lymphocyte count (RLC) and relative eosinophil count (REC)) with outcome after PD1 inhibition, we used the last differential blood count performed immediately prior to the first received dose of nivolumab.

Results: RMC, RNC and RLC did not have a prognostic impact on PFS, whereas higher WBC ≥ 7.76x10³/µl and lower REC<1.7% were associated with worse PFS in both univariate and multivariate analysis. We constructed a simple score to prognosticate PFS. By adding 1 point each for WBC ≥ 7.76x10³/µl and REC<1.7% to the score, we could clearly differentiate a low (score=0), intermediate (score=1) and high risk (score=2) group for disease progression (p<0.001). Only one PFS event occurred in the best prognostic group (n=10, median PFS (days): 365 [129-NA]) and 7 out of 9 patients in intermediate (median PFS (days): 197 [50-NA]). Evaluation of best response achieved according to the initial risk score showed a trend towards higher CR-rates in low risk group, but was not significant.

Figure 1.

Summary/Conclusions: Our simple prognostic model, mainly characterized by a normal to high REC, robustly discriminates three risk groups for PFS. Almost all patients in the low risk group achieved a durable remission without disease progression throughout the study period, despite often achieving just a partial response. In contrast, high-risk patients often progressed quickly despite initially achieving a partial or complete response. Further validation of this score which is easily available from routine clinical parameters in a larger cohort of patients and further investigation of its potential predictive impact is needed. Moreover, efforts to clearly understand a possible mechanistic role of eosinophils in cHL patients treated with PD1-inhibition are warranted.

THE PROGNOSTIC SIGNIFICANCE OF BETA-2 MICROglobulin (B2M) LEVELS IN PATIENTS WITH Hodgkin lymphoma (HL) TREATED WITH ABVD OR EQUIVALENT (ABVDEQ) CHEMOTHERAPY OR COMBINED IMMUNE CHECKPOINT BLOCKADE (ICB) THERAPY


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Background: The prognosis of HL primarily depends on clinical stage (CS) as well as limited-stage risk classification schemes and the International Prognostic Score (IPS), both of which were calculated based on the patient’s age, CS, and a wide variety of other factors including type of HL, tumor burden, and response to treatment. The main therapy in early-stage HL is ABVD or ABVD-like chemotherapy (ABVDEQ). However, the prognosis in young HL patients remains largely unknown. Recently, efforts have been made to assess the role of the b2m in HL, highlighting the significance of the cut-off used to define “high” levels. Its significance is more pronounced in early stage disease. The optimal cut-off for the evaluation of serum b2m in HL may be stage-dependent and needed. Moreover, efforts to clearly understand a possible mechanistic role of eosinophils in cHL patients treated with PD1-inhibition are warranted.

Aims: Our aim was to investigate the prognostic significance of serum b2m levels in HL.

Methods: We analyzed 864 patients with HL treated with ABVD/Deq CT/CMT (1990-2016) and selected solely based on the availability of pretreatment b2m levels. B2m [P1] levels (upper normal limit 2.4mg/L) were analyzed according to other baseline features and prognostic factors as well as according to the outcome. Freedom From Progression (FFP) was defined as time between treatment initiation and treatment failure (primary refractoriness, PR with switch to alternative CT or relapse); deaths of unrelated causes were censored. Overall Survival (OS) was measured from treatment initiation to death of any cause. ROC curves and sequential cut-offs (1.8-3.5 by 0.1 increments) were used to explore the potential impact of b2m on FFP and OS.

Results: The median follow-up for currently living patients was 88 months. Univariate Analysis: FFP was significantly inferior in patients with higher b2m at all tested cut-off points. At 2.4mg/L (normal versus elevated) the 10-year FFP was 81% vs 71% (p=0.003). However, the best cut-off was the observed median value of this series, calculated at 2.1mg/L, with 10-year FFP of 84% vs 71% (p=0.0001). In early stages (IA/IIIA) significant results were obtained at cut-offs between 1.8 and 2.1mg/L. The best cut-off was 1.9mg/L, a close approximation of the median b2m level of early stage patients, with 10-year FFP of 89% vs 78% (p=0.003). In advanced stages, none of the cut-offs yielded statistically significant results (borderline at 2.0mg/L, 10-year FFP 77% vs 67%, p=0.057). Multivariate Analysis: B2m levels remained significant for FFP after adjustment for IPS factors, ESR and B-symptoms at both 2.1mg/L and 2.4mg/L cut-offs (hazard ratio (HR) 1.78, p=0.001 and 1.41, p=0.04 respectively) in the whole series of 864 patients. In early stages, b2m was a significant predictor of FFP at the cut-offs of 1.9mg/L and 2.1mg/L (HR 2.00, p=0.01 and 1.83, p=0.02 respectively), but only borderline at the cut-off of 2.4mg/L (HR 1.65, p=0.07). In advanced stages, b2m emerged as an independent prognostic factor for FFP at the cut-off of 2.2mg/L (HR 1.59, p=0.046 despite the lack of significance in univariate analysis), but was not significant at the 2.4mg/L cut-off. The 10-year OS was lower in patients with high b2m levels (10-year rates 91% vs 76%, p<0.0001).

Summary/Conclusions: Higher serum b2m emerged as a significant independent predictor of FFP for the cutoff of 2.0mg/L for the whole series and 1.9mg/L for early-stage patients. The prognostic significance in advanced stages was weaker (best cut-off 2.2mg/L). Serum b2m was also highly predictive of OS. This is by far the largest report on the prognostic significance of b2m in HL, highlighting the significance of the cut-off used to define “high” levels. Its significance is more pronounced in early stage disease. The optimal cut-off for the evaluation of serum b2m in HL may be stage-dependent and appear to lie between 1.9 and 2.2mg/L, thus performing better than a “normal versus high” evaluation (cut-off 2.4mg/L).

THE PREDICTIVE VALUE OF INTERIM PET-CT IN ELDERLY PATIENTS WITH HODGKIN LYMPHOMA

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Background: The prognostic value of interim PET-CT in elderly patients with r/r HL is yet controversial. Between 1993 and 2016, several reports from other groups have yielded heterogeneous results in small- to medium-sized patient series of no more than 220 patients, frequently under variable treatment.

Aims: Our aim was to investigate the predictive significance of interim PET-CT in elderly patients with HL.
Background: Hodgkin lymphoma (HL), a disease of mostly young patients, also peaks in the elderly. Despite the profound improvement in the clinical outcome of young patients, in the elderly, 5 year overall survival (OS) is estimated at only 40-55%. Interim PET-CT (iPET), known to be highly predictive for progression free survival (PFS) in young patients with HL, has not been sufficiently validated in elderly patients, nor have many other outcome predictors in HL of the elderly. 

Aims: The objective of the present study was to evaluate the significance of iPET in elderly patients with HL.

Methods: All consecutive patients (age ≥60) diagnosed with HL between 1998-2016 were retrospectively reviewed in this multi-center study. Baseline characteristics as well as PET-CT results at diagnosis, interim analysis and end of treatment (EOT) were recorded and analyzed. PET-CT results were classified as no evidence of disease (NED), partial response (PR), stable disease (SD) and progressive disease (PD).

Results: Ninety five patients from 5 centers were identified. Median age was 71 (range 60-89) years. Subtype was nodular sclerosis in 48% and mixed cellularity in 23%. Sixty three (69%) patients had advanced disease and mean international prognostic score (IPS) was 3.5±1.4. Fifty nine (63%) patients received first line treatment with ABVD, in 13 (14%) chemotherapy was followed by involved field radiotherapy. At EOT, sixty seven (82%) patients achieved OR (7% achieved PR, 10 (11%) were primary refractory and 2 (2%) died during treatment. Fifteen (16%) patients experienced relapse. Five years PFS and OS were 56% and 78%, respectively. ABVD treated patients had 5 year PFS and OS of 59% and 82% as opposed to 48% and 68% for all other regimens, but these differences were not statistically significant. Seventy two (76%) patients who underwent ABVD and iPET, 50 patients had NED on iPET; 20 had PR, 1 SD and 1 PD. NED-iPET was achieved in 47/70 (68%) patients who had NED-iPET, 12/21 (58%) patients who had PR-iPET and none of the patients with SD/PD-iPET (<0.01). In patients with either NED or PR on iPET, relapse occurred in 11 (15%) patients and 5 year PFS and OS were 82% and 95%, respectively. The 5 year PFS of these patients differed according to the depth of response on iPET - 69% vs 45%, (p=0.02, fig.1) in patients achieving NED vs PR, while 5 year OS did not reach statistical significance, 90% vs 71% (p=0.08). Restricted analysis, evaluating only 59 patients who were treated with ABVD, showed similar results with 94% of NED-iPET vs 45% of PR-iPET achieving NED on EOT-PET (p<0.01). Outcome differed according to the depth of response in iPET with 5 year PFS rates of 74% vs 34%, in patients achieving NED vs PR, respectively (p=0.01). 5 year OS rates were 92% vs 76%, in patients achieving NED vs PR (p=0.1).

E1122
HIGH-DOSE BENDAMUSTINE PLUS BRENTUXIMAB COMBINATION IS EFFECTIVE AND HAS A FAVOURABLE TOXICITY PROFILE IN THE TREATMENT OF REFRACTORY AND RELAPSED HODGKIN LYMPHOMA

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Background: The management of patients with refractory or relapsed Hodgkin lymphoma (HL), especially after autologous stem cell transplantation (ASCT), remains controversial. Bendamustine has demonstrated efficacy in several lymphoproliferative disorders but limited data are available regarding the schedule in patients with HL, in particular its dosage and the possible combinations for a synergistic effect. Brentuximab Vedotin is a CD30-directed antibody-drug conjugate, currently approved for the treatment of relapsed or refractory HL.

Aims: The objective of this retrospective observational trial was to evaluate efficacy and safety of salvage cytotoxic regimens in patients with refractory and/or relapsed HL. Three different regimens were evaluated.

Methods: From May 2011 to December 2016, 32 consecutive patients (19 M/13 F) with a median age of 31.7 years (range, 16-73) received a salvage regimen after failure of ASCT. Patients were by chance assigned to one of these three arms: standard dose bendamustine (90mg/m²q2w) days 1 and 2 plus standard DHAP schedule (every 4 weeks) x 3 cycles (Arm A, n= 10 cases), brentuximab single agent 1.8mg/kg (every 3 weeks) x 4-8 cycles (Arm B, n= 11 cases), high dose bendamustine (120mg/q2w) days 1 and 2 plus brentuximab 1.8mg/kg (day 3) x 4-6 cycles (Arm C, n= 11 cases). Each cycle in arm C was repeated every 28 days and growth factor support was systematically administered, in association with antimicrobial prophylaxis. The treatment efficacy in each arm was evaluated according to Revised Response Criteria for Malignant Lymphoma by Cheson et al. Adverse event occurrence was recorded and classified for type and grade using NCI-CTCAE criteria (v 4.0).

Results: In arm A, the overall response rate (ORR) was 40% (4/10 patients), with 4 (40%) complete remission (CR) and 6 (60%) progressive disease (PD). Hematological toxicity was grade 3 thrombocytopenia in 4 patients (40%) and bone marrow aplasia in 1 patient (10%); extra-hematological toxicity was grade 3-4 neutropenia in 4 patients (40%) and grade 1 in 3 patients (30%), in arm B, ORR was 63.6% (7/11 patients), with 5 (45%) CR, 2 (18%) partial response (PR) and 4 (36%) PD. Hematological toxicity was grade 2 neutropenia in 4 patients (36%), extra-hematological toxicity was grade 3 neuropathy in 2 patients (18%). In arm C, ORR was 100% (11/11 patients), with 11 CR followed by SCT (second autologous transplant, 6 cases; and haploidentical transplant, 5 cases) with persistence of complete remission in all patients at a median follow-up of 33.4 months (range, 12-60). Hematological toxicity was grade 3 thrombocytopenia in 4 patients (36.3%); extra-hematological toxicities were increase of transaminases (grade 2), in 2 (18%) patients (27%) and cytomegalovirus (CMV) reactivation in 2 patients (18%), treated successfully with valganciclovir. Three patients had fever during infusion at first cycle, together with a skin rash, managed with corticosteroid injections, and a successful antihistamine plus corticosteroid prophylaxis in the next cycles of treatment.
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Background: In the last decades, Hodgkin and Non-Hodgkin Lymphoma (HL- NHL) therapies have resulted in high cure rates and increased survival. However, there are patients with HL who experience late toxicities, such as, gonadal toxicity that can result in permanent sterility.

Aims: to evaluate different aspects of fertility (menstrual status, pregnancy, and menopause) in women with HL and NHL in reproductive age before and after chemotherapy.

Methods: By a phone interview we administered a questionnaire to the patients. The interview was composed of questions concerning reproduction (progeny, menses and abortion) and also menopausal status. The analyses were made using data collected in a cohort 109 women patients from two Italian hematologic centers. Statistical analysis was carried out in Graphpad system, data were compared by the chi-square (P value <0.05) to be considered statistically significant.

Results: The median age (in years) at the time of the treatment was 31 (range 16-49), 69/109 (63%) had HL and 40/109 (37%) NHL, 74/109 [ES1] (64%) of the patients had a stage I-II. All HL patients were treated with ABVD, whereas the therapy in only 27% of NHL patients was R-CHOP (20%) or similar regimens (16%), respectively. Radiotherapy was delivered to the 62/109 (57%) of the sample. Complete Remission (CR) was obtained by the 101/109 (93%) and only 16/101 (16%) relapsed. Considering the gynecologic history of the patients there were no statistically significant difference between the regularity of menses and the event of an abortion pre and post treatment. As for pregnancies, 35% of patients had children before therapy and 17% after. Among these 109 patients, 68/109 (62%) received gonadotropin-releasing hormone (GnRH) analogues and/or oral contraception, while 41 (38%) were not treated with hormonal therapy. Among the 68 patients who received hormonal therapy regular menses recovered in 51/68 (75%) while in those of the control group a recover of menses was observed in 20/41 (48%). This difference was statistically significant (P<0.05). The same was observed as for early menopause. In this case excluding patients who had a natural menopause, a lower cases of early menopause was observed in those who received hormone therapy (8/68, 12% vs 30/41, 73% of the control group P=0.05). Considering only the 81/109 (74%) patients who had regular menses after chemotherapy, 61/81 (75%) received hormonal therapy and 20/81 (25%) were not treated with hormonal therapy. Before treatment for lymphoma, 16% of patients belonging to the hormonal group had pregnancies versus 45% of the control group (P=0.05). Following therapy, pregnancies were observed in 23% of those receiving hormonal therapy vs 5% of the control group (P=0.05).

Summary/Conclusions: The use of hormonal therapy is fundamental not only to favor of pregnancies and motherhood but in particular to avoid the consequences of an irregular cycle or an early menopause with its symptoms and clinical implications.

E1124
25(OH) VITAMIN D SERUM LEVELS IN Hodgkin Lymphoma

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Background: Vitamin D has pleiotropic effects on cellular differentiation, proliferation, apoptosis and angiogenesis in addition to maintaining serum calcium and skeletal homeostasis. Several studies suggest that low serum 25(OH) vitamin D levels may be associated with inferior outcome in solid tumors as colorectal and breast cancer, and in Non-Hodgkin lymphomas [Drake et al, J Clin Oncol 2010; 28:4191] as diffuse large B cell lymphoma [Bittenbring et al, J Clin Oncol 2014; 32:3243], and follicular lymphoma [Kelly 2010; 28:4191] as diffuse large B cell lymphoma [Bittenbring et al 2001 to November 2007 received a treatment based on ABVD with/without involved-field radiotherapy (IFRT). Treatment was modulated according to the stage. The 9 patients with stages I and II received 4 courses of ABVD plus IFRT, while 3 patients in stages III or IV received 6 cycles of ABVD. The subsequent 12 patients (diagnosis from December 2007 to July 2014) received R (375mg/m2) alone or combined with ABVD. The stage-adapted strategy of therapy was applied for these patients, as well. The 5 patients with early favourable disease according to the stage and other validated prognostic factors, received R as single agent (once per week for four consecutive weeks) followed by R maintenance (MR) (once every three months for 2 years); the 2 patients with early unfavorable stage were treated with R (once per month on day 1) plus 4 cycles of ABVD, while the remaining 5 advanced stage patients received R (on day 1 and 15) plus ABVD for 6 cycles. The primary end-point was DFS rate, and secondary end-points were ORR and treatment-related toxicity evaluation.

Figure 1.

Results: At final restaging, 4 weeks after the cycle of treatment or completion of IFRT, 23/24 patients (95.8%) were in CR while one patient showed refractory disease and was addressed to rescue therapy with autologous hematopoietic
stem cell transplantation (ASCT). Patients treated with R alone or R+ABVD had better DFS (p=0.04) than those treated with ABVD with/without IFRT. Specifically, the year Kaplan-Meier estimates for DFS were 100% for the R treated group versus 50% for those treated with ABVD with/without IFRT. Four patients in the latter group, showed insufficient response to the therapy: 1 refractory disease in the early stage group and 3 recurrent diseases in the advanced stage group were recorded. The median follow-up time of the entire cohort of patients was 4.3 years (range, 0.5-8.2 years). Over the study period, one patient died for infectious pneumonitis due to severe neutropenia following the last cycle of R-ABVD. Of the 9 patients treated with addition of IFRT, adverse events regarded mainly thyroid (4), bone (2), lung (1) and salivary glands (1). 

Summary/Conclusions: Our results confirm the value of R in NLPHL and show that R induction and maintenance combined with chemotherapy only in the presence of risk factors or in more advanced stages give excellent treatment results in respect-chemotherapeutic radio-chemotherapy either in term of ORR and of DFS while sparing long term toxicity usually seen in patients affected by classical HL who receive chemo and irradiation.

E1126

CASE-BASED LEARNING IN CONTINUING EDUCATION: IMPROVING HEMATOLOGIST/ONCOLOGIST EVIDENCE-BASED DECISIONS FOR PREVENTING HODGKIN LYMPHOMA POST-TRANSPLANT RELPASE

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Background: Several prognostic factors have been identified as associated with a higher rate of relapse after autologous stem cell transplantation (ASCT) for patients with Hodgkin lymphoma (HL). Due to the rarity of this disease, many hematologists/oncologists (hem/oncs), especially those in the community setting, lack experience to correctly identifying patients who may be at risk of post-transplant relapse. Proper risk assessment and understanding of treatment options in the pre- and post-transplant setting are critical to ensure optimal longer progression-free survival for qualified patients.

Aims: Underlying clinical practice gaps and educational needs were identified, and a study was conducted to determine whether an online, case-based educational intervention could improve knowledge, competence, and confidence of hem/oncs in managing patients with HL.

Methods: The educational format presented patient case scenarios (2) followed by a series of 4-5 questions that “tested” learner knowledge and competence before delivering the education focused on the optimal approach to the case using evidence-based medicine. Case questions assessed degree of patient risk for disease relapse or progression prior to ASCT and consolidation strategies, taking into consideration patients’ prior received therapies. To assess educational effectiveness, participants served as their own controls by responding to a series of same questions again after (post-assessment) exposure to the intervention. The median follow-up time of the entire cohort of patients was 4.3 years (range, 0.5-8.2 years). Over the study period, one patient died for infectious pneumonitis due to severe neutropenia following the last cycle of R-ABVD. Of the 9 patients treated with addition of IFRT, adverse events regarded mainly thyroid (4), bone (2), lung (1) and salivary glands (1).

Summary/Conclusions: Our results confirm the value of R in NLPHL and show that R induction and maintenance combined with chemotherapy only in the presence of risk factors or in more advanced stages give excellent treatment results in respect-chemotherapeutic radio-chemotherapy either in term of ORR and of DFS while sparing long term toxicity usually seen in patients affected by classical HL who receive chemo and irradiation.

E1127

QUANTITATIVE PET PARAMETERS PREDICTS OUTCOME IN PATIENTS WITH HODGKIN'S LYMPHOMA

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Background: Positron emission tomography [18F] fluorodeoxyglucose (FDG-PET) has emerged as the standard response assessment after 1st line therapy for classical Hodgkin’s lymphoma (HL). Quantitative PET parameters are not well established as a predictive factor for disease progression or survival in HL.

Aims: Thus, the aim of this study was to test the hypothesis that tumor burden characterized by mean standardized uptake value (SUVmean), maximum SUV (SUVmax), metabolic tumor volume (MTV) and total lesion glycolysis (TLG) could be independent prognostic factors.

Methods: We analyzed the relation of absolute value PET parameters, negative predictive value (negative PET scan and no treatment failure, NPV) and positive predictive value (positive PET scan and treatment failure, PPV) with event-free survival (EFS) or overall survival (OS). Quantitative PET parameters of the baseline (PET-1), interim (PET-2) and end of treatment (PET-3) PET-CT scans were investigated in the retrospective study. MTV was computed by using the 41% maximum SUV thresholding method, and the optical cut-off for survival prediction was determined.

Results: Thirty one patients with HL with a stage II-I–51.6%, III-IV–48.4% consecutively admitted from April 2009 to December 2016, by 5 Ukrainian hematological centers were included in the analysis. Patients were staged at baseline, after 2-4 cycles of chemotherapy with PET/CT and at the end of chemotherapy. All patients were treated with ABVD, BEACOOP-14/esc. All 31 patients achieved CR or PR and 67.7% had a negative PET-2, while 16.3% had a positive PET-2. Patients with negative PET-2 and positive PET-2 had CR rates of 64.5% and 12.1%, respectively, which yielded a PPV of 26% and NPV of 74%. ROC analysis revealed that PPV and NPV are an important markers associated with EFS in patients with HL (Se=100%; Sp=100%; AUC=1.0). 3-year EFS was 100% for NPV patients and 12% for PPV patients, which was statistically significant (p<0.01). The median follow-up time of the entire cohort of patients was 4.3 years (range, 0.5-8.2 years). Over the study period, one patient died for infectious pneumonitis due to severe neutropenia following the last cycle of R-ABVD. Of the 9 patients treated with addition of IFRT, adverse events regarded mainly thyroid (4), bone (2), lung (1) and salivary glands (1).
Indolent Non-Hodgkin lymphoma - Clinical

E1128

Abstract withdrawn.

E1129

BIOMARKER ANALYSIS OF PATIENTS WITH FOLLICULAR LYMPHOMA TREATED WITH IBRUTINIB IN THE PHASE 2 DAWN STUDY


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Background: Ibrutinib, a first-in-class, oral, covalent inhibitor of Bruton's tyrosine kinase, has demonstrated robust clinical activity and is approved in various B-cell non-Hodgkin's lymphomas. To assess the efficacy and safety of ibrutinib in patients (pts) with follicular lymphoma (FL), the DAWN study (FLR2002, NCCT1779791) investigated single-agent ibrutinib in chemotherapy (CIT)-refractory FL pts. Ibrutinib may exert immune-modulatory effects on T-cell activity via inhibition of ITK, a key regulator of T-cell activity, possibly through (CIT)-refractory FL pts. Ibrutinib may exert immune-modulatory effects on T-cells, chemokines, and cytokines in ibritutinib-treated CIT-FL pts.

Aims: To determine the effect of ibrutinib on circulating T-cells, chemokines, and cytokines in ibritutinib-treated CIT-FL pts.

Methods: The DAWN study was an open-label, multicenter, single-arm, phase 2 study of ibrutinib in pts with CIT-refractory (i.e., ≥3 prior lines of chemotherapy and progressive disease [PD]) ≤12 months after last dose of a CIT regimen. All pts received ibrutinib (560mg QD) on a 21-day cycle until PD or unacceptable toxicity. The primary end point was Independent Review Committee (IRC)-assessed overall response rate (ORR) (complete response [CR] + partial response [PR]). Flow cytometry assessed T-cell subpopulations at baseline (C1D1) and at cycle 3 (C3D1) for 57 pts (14 responders and 43 nonresponders); cytokine and chemokine analyses were performed at C1D1 and at cycle 2 (C2D1) for 50 pts (21 responders and 29 nonresponders).

Results: Results from the DAWN study have been presented previously (Gopal A. et al. ASH 2016). Briefly, 110 pts with a median age of 61.5 years and a median of 3 prior therapies were enrolled. Ibrutinib achieved an ORR of 20.9% (CR rate, 10.9%) and a median duration of response of 19.4 months. Flow cytometry analysis revealed significant downregulation of CD4+CD25+CD127-Tregs at C3D1 in 14 responders (CR + PR, mean decrease 17 to 12.9% CD4, p=0.02) but not in 43 nonresponders (SD + PD, 11.5 to 10.4% CD4, p=0.17).

Conversely, the chemokines IFN-γ-induced protein 10 (IP-10) and monocyte-macrophage chemotactic protein 3 (MCP-3) were decreased in responders but increased in nonresponders (p=0.022 and p=0.016, respectively).

Summary/Conclusions: Here we show immunomodulatory effects of ibrutinib in pts with CIT-refractory FL, which may be related to response to therapy. In responders, pts at early time points, downregulation of Tregs was observed, along with increases in Th1-associated cytokines IFN-γ and IL-12. This shift in T-cell polarization may be linked to the antitumor response; in nonresponders, these cytokines were decreased but Tregs were not. Chemokine changes observed also indicate variation in chemotraction of T-cells and monocytes/macrophages. These data suggest that immunomodulatory effects of ibrutinib could play a role in its antitumor activity in FL, so combinates with other immune-oncology therapies may prove beneficial.

E1130

DYNAMO: THE CLINICAL ACTIVITY OF DUVELISIB IN PATIENTS WITH DOUBLE-REFRACTORY SMALL LYMPHOCYTIC LYMPHOMA IN A PHASE 2 STUDY

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Background: Duvelisib is an oral, dual inhibitor of PI3K-δ,γ in development for the treatment of hematologic malignancies. DYNAMO is a Phase 2 study to evaluate the safety and efficacy of duvelisib in a double refractory iNHL population, which included 28 patients (pts) with small lymphocytic lymphoma (SLL) or chronic lymphocytic leukemia (CLL).

Aims: The primary objective was to evaluate the antitumor activity of duvelisib monotherapy in pts whose disease is refractory to rituximab and to either chemotherapy or RIT, with an additional objective to further characterize the safety duvelisib.

Methods: DYNAMO is an open-label, single-arm, safety, and efficacy study in patients (pts) with FL, small lymphocytic lymphoma (SLL), or marginal zone lymphoma (MZL), whose disease is double refractory to rituximab (monotherapy or in combination) and to chemotherapy or radioimmunotherapy. Pts received duvelisib 25mg BID in 28-day treatment cycles until disease progression or unacceptable toxicity. The primary endpoint is overall response rate (ORR) as assessed by an independent review committee (IRC) per revised IWG criteria. Secondary endpoints include duration of response (DoR), progression-free survival (PFS), overall survival (OS), time to response (TTR), adverse events (AEs), and changes in safety laboratory values. Pneumocystis jiroveci pneumonia (PJP) prophylaxis was mandated for all pts.

Results: 129 pts with iNHL were treated on study. Of these, 28 pts with SLL received duvelisib with a median duration of exposure of 9 mo. (range 6.5-12). Median age was 65 years; 68% were male. Most SLL pts had either histologic (61%) or functional (39%) disease. Median time from last anticancer therapy to first dose of duvelisib was 3 months. SLL pts received a median of 3 prior anticancer regimens (range: 1-8); 43% of pts received ≥4 prior anticancer regimens, 29% ≥6 regimens. The ORR for SLL pts was 68% (95% CI: 48, 84) per IRC assessment. All responses (19) were PRs. Four (14%) pts had a best response of SD and 3 (11%) pts had a best response of PD. 2 pts were unacceptable for response. Per Investigator assessment, the ORR was 79% (including 1 CR). Median time to IRC response was 1.9 months (range 1.4-5.5). 93% of pts had a reduction in nodal target

Figure 1.
lesions. Among the 19 SLL pts with a response per IRC, the median DOR was 9.8 months. The median PFS among all SLL pts was 11.3 months, while the median OS was not reached. The estimated probability of survival at 12 months was 83.9%. Among all pts treated (n=129), AEs were mostly Gr 1-2. Most common ≥ Gr 3 AEs were transient cytopenias (neutropenia [23%], anaemia [12%], and thrombocytopenia [10%]), and diarrhoea (15%). 4 SLL pts had SAEs that led to discontinuation of duvelisib: NSCLC, neuroendocrine carcinoma of the skin, pseudomembranous colitis, and pneumonia. Two SLL pts has a fatal AE, 1 pneumonia and 1 viral infection.

Summary/Conclusions: In DYNAMO, duvelisib showed clinical activity in a double-refractory SLL population (68% ORR, median DOR 9.9 mo., 93% with a reduction in target lesions). Duvelisib was generally well tolerated, with a manageable safety profile with appropriate risk mitigation. Duvelisib monotherapy appears to have a favorable benefit-risk profile in double refractory SLL.

Updated clinical data will be available at the time of presentation.

E1131
Abstract withdrawn.

E1132
WALDENSTROM MACROGLOBULINAEMIA: UK REAL WORLD EXPERIENCE
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Background: There are few randomised controlled trials in Waldenström macroglobulinemia (WM) due to its rarity and indolent nature. As a result, there is no standard treatment approach and management is variable.

Aims: The aim of this retrospective study was to review “real world” management of WM in the UK and correlate this with survival outcomes.

Methods: All patients with a diagnosis of WM seen at ULCH between 01/07/2002 and 31/12/2016 were included. Patient characteristics, presenting features, lines of treatment, responses and overall outcome were recorded. The study was approved by the local Research Ethics Committee.

Results: Over the study period, 128 WM patients were identified (69 M/59 F), median age 60 yrs (range 34-89). Presenting symptoms included anaemia, n=33; neuropathy, n=19; fatigue, n=18; hyperviscosity symptoms, n=13; lymphadenopathy, n=6; progression from MGUS, n=5; B symptoms, n=5; other, n=28; unknown, n=55. Mutated MYD88 was seen in 59 of 72 cases analysed (82%). Of these 59 cases, 13 were CXCR4 mutated. IPSSWM was known in 150 cases of whom 64 were in low, 63 intermediate and 23 high risk groups. Median follow-up from first appointment was 64 months (range 0-394). The median number of lines of therapy was 2 (range 0-9). Dexamethasone, rituximab and cyclophosphamide (RDC) was given to 62 patients upfront, 52 had other cyclophosphamide containing regimens e.g. CHOP +/- rituximab, 29 had Chlorambucil-based regimen, 14 R-bendamustine, 15 fludarabine-based with a minority getting R-cladribine (5) or R-bortezomib (4), 9 pts had no treatment at data cut-off. Notably, DRC was given to 1 patient before 2009, 28% of patients between 2009 and 2013, and 41% from 2013. In the 149 cases with known responses to first line treatment, 11% achieved a CR (7 patients with R-CHOP, 4 DRC, 2 fludarabine containing regimen, and 3 patients other treatment), 63% PR VGRP, 21% no response or PD and 5% stopped due to toxicity. For the 52 patients who had DRC chemotherapy, median PFS was 61 months. Of those patients who had at least 3 lines of chemotherapy (n=62), median time between 1st and 2nd line treatment was 10 months, and between 2nd and 3rd line, 12 months. Transplants were performed on 28 patients after a median of 2 lines of chemotherapy. Median overall survival (OS) has not been reached in the 195 patients with available data. Stratifying by IPSSWM shows median OS for the low risk group has not been reached, 11 years for the intermediate risk and 9 years for the high risk group, P=0.29 (Figure). Patients had a significantly reduced OS if they developed Bing Neel syndrome or high grade transformation compared to other known complications of WM. Despite differences in chemotherapy strategies over the past two decades, there was no difference in outcome in patients treated before 2005, between 2005-2009, 2009-2013 and 2013 onwards. Of the 34 deceased patients, the cause of death was unknown in 3 cases, due to PD in 16 and other causes in 15 cases.

Summary/Conclusions: The management of patients with WM in this large case series reflects the variability of treatment given over time and also geographically. UCLH treats both a local and tertiary referral patient population, thus it is not completely typical. Survival data confirms the IPSSWM is likely to still differentiate patients into prognostic groups but the overall prognosis is better than when first published. With the advent of targeted therapies, it is imperative to perform randomised controlled trials and to collect data prospectively in order to elucidate the optimal management. To this end, a WM Biobank and Registry has been set up at our centre.

E1133
CLINICAL CHARACTERISTICS AND LONG-TERM RESULTS OF TREATMENT OF INDOLENT NON-HODGKIN’S LYMPHOMA ASSOCIATED WITH HEPATITIS C (IL + C)
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Background: According to the WHO classification (2008) hepatitis C virus is one of the causess of non-Hodgkin lymphoma. The incidence of chronic hepatitis C (HCV) in patients with indolent B-cell non-Hodgkin’s lymphoma (IL + C) is 15%. Diagnosis of hepatitis C related lymphoma (IL + C) is established in cases with history or present or former issue of chronic hepatitis C V (+) or positive tests with expression of proteins of hepatitis C virus. These proteins could be defined by immunochemistry (ICH).

Aims: The aim of this work was evaluation of the results of treatment of IL associated with hepatitis C in comparison with a control group of patients with IL without viral hepatitis markers.

Methods: The study included 107 patients with indolent lymphoma who were identified in the blood markers of hepatitis C.

Results: Histological types were follicular lymphoma - 74%, marginal zone lymphoma - 32%. The age of patients ranged from 28 to 82 years (median 50). Men / women ratio was 1: 1. Stage I + II were in 3%, III stage was in 24% of patients, IV stage was at 73% of patients. Primary extranodal lymphoma was diagnosed in 33% of patients. Extranodal lesions: splenic lesion in 53% of patients, liver injury - 21% of the patients, the bone marrow - 62% of patients. LDH ≥ 450 IU/l was at 76% cases, ALT ≥ 40 IU/l was at 82% of cases, albumin <35 g / l was at 31% of patients. 57 patients were treated with interferon and Ribavirin as a first-line treatment. Treatment lasted for 2 years after reaching the antitumor effect. 50 patients were treated with immunotherapy (CHOP, R-CVP) as a first-line treatment. Antiviral therapy was effective in 88% patients, immunotherapy was effective in 64% of patients. Median progression-free survival in patients with IL + C treated with antiviral treatment was 42 months, in patients with IL + C treated with immunotherapy - 19 months (p=0.0001). Five-year overall survival was 67% and 32%, respectively (p=0.0003). It was diagnosed disease relapses after immunotherapy in 39 patients. All the patients in the second-line was received antiviral treatment. The therapy was ongoing for 12-14 months. Of patients, one progression-free survival in relapsed lymphoma was 31 months.

Summary/Conclusions: Antiviral therapy in first-line and relapse of disease surpasses all the indicators of efficacy of treatment IL + HCV. In this category of patients preferred option is to conduct anti-viral treatment.

E1134
90Y-IBRITUMOMAB-TIUXETAN AS FIRST-LINE CONSOLIDATION IN COMPLETE RESPONSE FOLLICULAR LYMPHOMA PATIENTS. SINGLE CENTER ANALYSIS AFTER SIX YEARS MEDIAN FOLLOW-UP
M. Andrade-Campos1,*, N. Espinosa Lara2, P. Lievano Segundo3, L. Lopez4, T. Baringo5, P. Giraldo5
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Background: Follicular lymphoma (FL) accounts for around 22% of all non-Hodgkin lymphomas. His natural history is characterized by multiple relapses and progressively shorter response durations after every new line of therapy for this is desirable to offer the best first-line approach to each patient. In the current guidelines several first-line options are included: immunotherapy (Rituximab (R) x4 or Lenalidomide+/ R), immunochemotherapy (CHOP, RCVP, Bendamustine + R), radioimmunotherapy for elderly patients. Moving forward, the consolidation with radioimmunotherapy or extended dose immunotherapy

haematologica | 2017; 102(s2) | 465

Madrid, Spain, June 22 – 25, 2017
(R every 8 weeks for 4 or 12 doses) still appears as an optional part of the therapy (NCCN V3.2016). Radiomunotherapy with 90Ytrium-britimumab tiuxetan (90Y-IT) is available in our institution since 2006 and more than 100 patients have been treated with RIT since then. Here an institutional analysis focus in their use as consolidation is presented

**Aims:** To analyze the experience with 90Y-IT as a consolidation therapy in patients in CR after first-line therapy.

**Methods:** A retrospective analysis was performed including all the patients who have received RIT with 90Y-IT. Inclusion criteria were: patients 18 years or older with a grade 1-2a follicular lymphoma, RIT was received as a consolidation therapy in complete response (CR) after a first-line therapy. Demographic and follow-up data were included. International working group (IWG) criteria of response was used. Progression free survival (PFS) was calculated from the date of RIT to the date of a confirmed relapse according IWG criteria, overall survival (OS) was calculated from the FL diagnosis to the last contact.

**Results:** A total of 31 FL patients have received 90Y-IT been in CR after a first-line therapy and were included for the study. Mean age at diagnosis was 61.2 (29-86) years with a female predominance (19, 61.3% vs 12, 38.7%), 80.6% (26) with ECOG 0-1 and 19.4 ECOG 2. A third of them (10, 32.3%) were treated with follicular lymphoma (J Clin Oncol 2015;33:2616) or other chronic hematological malignancies (Blood 2009;114:1299; Blood 2016:128:902), few retired attempted to decipher the evolution of pts with WM, a cancer associated with delayed response to therapy in some pts.

**Aims:** To assess the prognostic role during the clinical course of initial interna
tional prognostic index (IPSSWM), response and progression (according to 6th International Workshop guidelines).

**Methods:** We took advantage of our continuously updated clinical database for reviewing a series of 114 symptomatic WM pts treated in our 2 institutions between 1993 and 2016 (median age 70, male/female ratio=1:1.9, high, low/intermediate and unfavorable IPSSWM in 57, 36 and 21 pts respectively).

Response rate after 1st line therapy was 70%. Sixty-two, 37 and 19 pts received a 2nd and 3rd and 4 to 6 lines of therapy respectively according to the 2nd International Workshop guidelines. Monitoring of serum monoclonal immunoglobulin concentration (SMIC) throughout the evolution of the disease was available in 106 pts. Informed consent was obtained according to the protocol submitted to the Ethics Committee of the Institution Review Board.

**Results:** Median survival after 1st line was estimated 79 months. It was esti
mated 69 and 65 months after 2nd line and 3rd line respectively. High IPSSWM (hiIPSSWM vs low/intermediate) retained prognostic value for survival after 1st treatment initiation (SAFTI, p=0.0005). However, plot of hazard function showed a decrease of hazard ratio over time with a departure from the proportional hazard hypothesis (Grambsch and Therneau test: p=0.053). Consequently, Dxy concordance index obtained in multiple landmarks analyses decreased from 0.27 to 0.12, during the first 6 years of follow-up. In Cox model of SAFTI with time dependent covariate, onset of response (whatever cut-off in SMIC) and progression had no prognostic value. By contrast, onset of progression and initiation of 2nd line therapy, retained prognostic values for SAFTI (p=0.0038 and p=0.004 respectively). Only 2 thresholds in SMIC defined a response status (observed between onset of response and progression) of prognostic value for SAFTI: namely >25% reduction in SMIC (i.e. minor response or better: p=0.041) and 50% (i.e. partial response or better: p=0.026). In similar Cox models with hiPSS-
WM, onset of progression (p=0.0034) and 2nd treatment initiation (p=0.0031) retained independent prognostic value beside hiIPSSWM (p<0.0026). Times elapsed from the initiation of 1st line therapy to 1st progression and to the initi-
atation of 2nd line therapy had no prognostic value for subsequent survival. In similar Cox model of survival after 2nd line therapy with time dependent covari-
ate no threshold in SMIC were found to be associated with a significant value of onset of response or response status. Neither onset of progression nor next treat-
ment initiation had significant prognostic value. Similar results were observed after the 3rd line of therapy.

**Summary/Conclusions:** The prognostic value of initial IPSSWM decreased in part during the first 6 years of evolution. Onset of progression and 2nd treat-
ment initiation provided additional prognostic information for predicting SAFTI. Therefore progression-free survival or time to next treatment may be satisfac-
tory surrogate endpoint of SAFTI in WM. Further international collaborative studies are mandatory for this purpose. Assessing response in advanced phase of the disease may require specific tools.

**E1135**

**ASSESSING RISK OVER TIME IN PATIENTS WITH SYMPTOMATIC WALDENSTROM MACROGLOBULINEMIA (WM). A STUDY ON 114 PATIENTS**

**Background:** Aims were to perform a retrospective study on a large series of patients with WM to assess the clinical impact of time dependent factors on disease progression.

**Methods:** We performed a retrospective study on a series of 114 WM patients treated in our institution from 1989 to 2016. The time to progression (TTP) was defined as the time from the initial diagnosis to disease progression. The study was approved by our institutional ethics board.

**Results:** Median age was 66 (50-81) years and 53 (47%) were men. The disease had been considered indolent in 82 (73%) for a median time of 5.5 (1-137) years, and aggressive in 32 (27%) for a median time of 0.5 (0.1-15) years. Median follow-up time was 64 (1-264) months. Median TTP was 74 (24-238) months. TTP according to the logistic regression model was related to age (p=0.040), sex (p=0.021), and time to diagnosis (p=0.018).

**Conclusion:** The time to diagnosis is an independent prognostic factor for TTP in WM.

**E1136**

**TIME TO NEXT TREATMENT ANALYSIS FOR EARLY AND ADVANCED STAGES OF MYCOSIS FUNGOIDES/SEZARY SYNDROME TREATED WITH BEXAROTENE AND PUVA IN COMBINATION**

**Background:** Bexarotene is a syntetic retinoid effective in early and advanced stages of Mycosis Fungoides (MF)/Sezary Syndrome (SS) both in monotherapy and combination schemes. Time to next treatment (TTNT) seems to be a clinically meaningful endpoint that incorporates both symptom control and disease progression. It has been investigated in few retrospective studies focusing on retinoids in monotherapy both in limited-stage and advanced stage MF, but up to now no data are available concerning the use of retinoids in combination.

**Aims:** To assess the prognostic role during the clinical course of initial interna
tional prognostic index (IPSSWM), response and progression (according to 6th International Workshop guidelines).

**Methods:** We took advantage of our continuously updated clinical database for reviewing a series of 114 symptomatic WM pts treated in our 2 institutions between 1993 and 2016 (median age 70, male/female ratio=1.9, high, low/intermediate and unfavorable IPSSWM in 57, 36 and 21 pts respectively).

Response rate after 1st line therapy was 70%. Sixty-two, 37 and 19 pts received a 2nd and 3rd and 4 to 6 lines of therapy respectively according to the 2nd International Workshop guidelines. Monitoring of serum monoclonal immunoglobulin concentration (SMIC) throughout the evolution of the disease was available in 106 pts. Informed consent was obtained according to the protocol submitted to the Ethics Committee of the Institution Review Board.

**Results:** Median survival after 1st line was estimated 79 months. It was esti
mated 69 and 65 months after 2nd line and 3rd line respectively. High IPSSWM (hiIPSSWM vs low/intermediate) retained prognostic value for survival after 1st treatment initiation (SAFTI, p=0.0005). However, plot of hazard function showed a decrease of hazard ratio over time with a departure from the proportional hazard hypothesis (Grambsch and Therneau test: p=0.053). Consequently, Dxy concordance index obtained in multiple landmarks analyses decreased from 0.27 to 0.12, during the first 6 years of follow-up. In Cox model of SAFTI with time dependent covariate, onset of response (whatever cut-off in SMIC) and progression had no prognostic value. By contrast, onset of progression and initiation of 2nd line therapy, retained prognostic values for SAFTI (p=0.0038 and p=0.004 respectively). Only 2 thresholds in SMIC defined a response status (observed between onset of response and progression) of prognostic value for SAFTI: namely >25% reduction in SMIC (i.e. minor response or better: p=0.041) and 50% (i.e. partial response or better: p=0.026). In similar Cox models with hiPSS-
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**Summary/Conclusions:** The prognostic value of initial IPSSWM decreased in part during the first 6 years of evolution. Onset of progression and 2nd treat-
ment initiation provided additional prognostic information for predicting SAFTI. Therefore progression-free survival or time to next treatment may be satisfac-
tory surrogate endpoint of SAFTI in WM. Further international collaborative studies are mandatory for this purpose. Assessing response in advanced phase of the disease may require specific tools.
Results: We enrolled 21 patients, 12 males and 9 females, with median age of 67 years (range, 30-77), of which 15 affected by early MF (13 with stage IB, 2 with stage IIA) and 7 by advanced disease (2 with stage IIB, 2 with stage IIIA, 1 with stage IIIB and 1 with stage IVA). Six patients had previously received PUVA therapy only, while fifteen patients had received other therapies. The protocol proved to be effective, well tolerated and able to induce an overall response of 55.6% at the end of induction phase (93.4% of early stage patients and 66.6% of advanced stage patients) and of 76.2% at the end of maintenance phase (86.7% of early stage patients and 14.2% of advanced stage patients). Median follow up for all patients was 85 months (6-118) with respectively 98 months (21-118) for early stages and 46 months (6-102) for advanced stages. For the entire cohort, median OS, PFS and TTNT were not reached; mean values of OS, PFS and TTNT were respectively, 105, 103 and 79 months, and median EFS was 58 months. For advanced stage patients, median OS, PFS, EFS and TTNT were 32, 29, 18 and 39 months respectively.

Summary/Conclusions: Our combination treatment seems to have superior TTNT compared to data published in the literature for PUVA and bexarotene used in monotherapy. When considering early and advanced MF, 66% of our patients are estimated to be free from further treatment at 2 years, a higher percentage compared to the results of Hughes et al. (Blood, 2015) for patients treated with PUVA (54.2%) or bexarotene (36.8%) as single agents. Moreover, TTNT seems to be longer in our study than in the study by Hanel et al (AJH 2016) on patients treated by retinoids in monotherapy, respectively 79 vs 60 months (mean TTNT values) in the early stages and 39 versus 9 months (median TTNT values) in the advanced stages. We believe that our results strongly suggest a synergistic or additive effect between PUVA and bexarotene compared to either agent alone in the treatment of both limited-stage and advanced stage MF.

E1137
PERIPHERAL BLOOD INVOLVEMENT IN PATIENTS WITH ADVANCED STAGE FOLLICULAR LYMPHOMA: CLINICAL-BIOLOGICAL CHARACTERISTICS AND PROGNOSTIC IMPACT

A. Rivas-Delgado1,*, L. Magnano 2, P. Mozas 1, I. Dlouhy 1, J. Rovira 1

AND PROGNOSTIC IMPACT

34x501 in the treatment of both limited-stage and advanced stage MF.

or additive effect between PUVA and bexarotene compared to either agent alone for the advanced stages. We believe that our results strongly suggest a synergistic Background:

Follicular lymphoma (FL) is the second most common type of non-Hodgkin’s lymphoma. While there are therapeutic options for patients with FL, it remains an incurable disease with conventional therapies. Furthermore, real-world treatment patterns for patients with FL are not well characterized in the literature. Aims: To characterize the real-world treatment patterns by line of therapy (LOT) for patients with FL in a large US-insured database.

Methods: Using the Optum integrated database, patients with FL were identified and included if 1) they were diagnosed with the International Classification of Diseases, Ninth Revision (ICD-9) codes 202.0 or 202.00 to 202.08 between January 2010 and December 2014; 2) their age was ≥18 years at the index date (defined as date of FL diagnosis); 3) they did not have any other primary cancers during the period from 3 years prior to index date up to 1 month post-index date; and 4) they had continuous insurance coverage for 365 days prior to index date. All reporting was done using descriptive statistics.

Table 1.

<table>
<thead>
<tr>
<th>Therapy</th>
<th>LOT 1</th>
<th>LOT 2</th>
<th>LOT 3</th>
<th>All LOTs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rituximab monotherapy</td>
<td>21.3%</td>
<td>20.9%</td>
<td>21.7%</td>
<td>21.5%</td>
</tr>
<tr>
<td>Rituximab-containing regimens</td>
<td>78.7%</td>
<td>79.1%</td>
<td>78.3%</td>
<td>78.5%</td>
</tr>
<tr>
<td>Rituximab-doxorubicin-bleomycin-vincristine-prednisone (R-CHOP)</td>
<td>10.2%</td>
<td>11.4%</td>
<td>10.1%</td>
<td>10.6%</td>
</tr>
<tr>
<td>Rituximab-doxorubicin-bleomycin-vincristine-prednisone without rituximab (R-CHOP-R)</td>
<td>9.7%</td>
<td>8.6%</td>
<td>9.9%</td>
<td>9.6%</td>
</tr>
<tr>
<td>Rituximab-containing regimens without rituximab</td>
<td>8.9%</td>
<td>7.9%</td>
<td>9.2%</td>
<td>8.7%</td>
</tr>
<tr>
<td>Other chemotherapy</td>
<td>0.1%</td>
<td>0.1%</td>
<td>0.1%</td>
<td>0.1%</td>
</tr>
</tbody>
</table>

Results: A total of 2569 patients with FL met the inclusion criteria and were included in the analysis. In this cohort, the mean age was 60 years; 51% were male; 72% were Caucasian, 5% African American, 2% Asian, and 20% other. The median duration of follow-up was 610 days. Across all LOTs, 1180 patients (46%) had at least one National Comprehensive Cancer Network (NCCN) guideline-recommended treatment for FL, and 153 patients (6%) had only 21% of patients in the early stage group. Across all LOTs, the use of other FL treatments was very low, including rituximab-cyclophosphamide-vincristine-
E1139

A PHASE 1 STUDY EVALUATING THE SAFETY AND PHARMACOKINETICS (PK) OF VENEToclAX (VEN) IN JAPANESE PATIENTS (PTS) WITH NON-HODGKIN LYMPHOMA (NHL) AND MULTIPLE MYELOMA (MM)

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Background: The antiapoptotic protein BCL-2 is commonly overexpressed in hematologic malignancies. VEN is a potent, selective, orally bioavailable BCL-2 inhibitor that has demonstrated acceptable safety and antitumor activity in NHL and MM pts.

Aims: To evaluate the PK profile, and preliminary antitumor activity of single-agent VEN in Japanese pts with NHL or MM.

Methods: Phase 1 open-label, dose-escalation study of VEN in Japanese pts with relapsed or refractory (R/R) NHL or MM (NCT02265731). Dose escalation followed a 3+3 design. After a 2-week ramp-up period with weekly dose escalation, VEN was administered at daily final doses of 300, 600, 900, or 1200mg on 21-day cycles until progression. All pts received t = 150 days before the first VEN dose and before each dose escalation. Adverse events (AEs) were assessed by NCI CTCAE v4.0. Dose-limiting toxicities (DLTs) were determined during the ramp-up period and during cycle 1. Responses were assessed by 2007 IWG (NHL) or 2006 IMWG (MM) criteria. T-cell evaluation was performed in all pts.

Results: As of January 19, 2017, 20 pts (50% male; median age 65 years [39–81]) have been enrolled: 3 pts in the 300-mg, 7 pts in the 600-mg, 7 pts in the 900-mg, and 3 pts in the 1200-mg VEN dose cohorts. Eighteen (90%) pts had NHL (stage III/IV, n=14), including 11 with follicular lymphoma (FL), 6 with diffuse large B-cell lymphoma (DLBCL), and 1 with concurrent FL+DLBCL; 2 (10%) pts had MM at diagnosis. Treatment-emergent AEs (all grades) ≥ 20% pts were lymphopenia (80%), neutropenia (60%), leukopenia (50%), and anemia (25%), and non-hematologic toxicities including nausea (55%), vomiting, diarrhea, and nasopharyngitis (30%) each. Grade ≥ 3 treatment-related AEs were lymphopenia (45%), neutropenia (40%), and leukopenia (30%). One pt in the 1200-mg VEN dose cohort received reduced doses of VEN (150mg/day) due to experiencing dose-limiting lymphopenia (DLT) after receiving 2 doses of 100-mg VEN on day 2 of the dose-ramp-up period. One DLBCL pt died while on study due to disease progression. No TLS events were reported.

E1140

A SIMPLIFIED APPROACH IN THE ASSESSMENT OF T-CELL CLONALITY BY FLOW CYTOMETRY

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Background: T-cell lymphoproliferative disorders are amongst the most challenging diagnoses in haematology. Flow cytometric T-cell receptor (TCR)-Vβ repertoire analysis (TCR-Vβ-R) is a sensitive method for detection of T-cell clonality; however, the assay is cumbersome owing to the required eight-part analyses that limit its clinical utility.

Aims: Here we describe a simplified flow cytometric method utilising a monoclonal antibody that targets the T-cell receptor β constant domain 1 (TRBC1). The cβ TCR is a pan T-cell antigen, expressed on >90% of T-cell lymphomas and all normal T-cells. A feature of the TCR is that the β-constant region comprises 2 functionally identical genes: TRBC1 and TRBC2. Each T-cell expresses only one of these. Consequently, normal T-cells will be a mixture of individual cells expressing either TRBC1 or 2, while a clonal T-cell disorders will exclusively express TRBC1 or 2.

Methods: Using multiparameter flow cytometry we assessed the expression of Jovi-1 in normal donors (n=19), T-cell leukaemia cell line (n=1), T-GL (n=9), T-NHL (n=3), Sezary syndrome (n=3) and patients with reactive lymphocytosis (n=4). A comparison of Jovi-1 and T-GL-Vβ-R was also performed to compare the two approaches.

Results: Jovi-1 expression within the CD4 and CD8 compartments of T-cells in normal donors was a median of 42.6% (range 33.7%-49%) and 36.4% (range 22.3%-48.5%) respectively. The T-cell line, Jurkat was exclusively positive for Jovi-1. Of the 9 patients with T-LGL, 7 patients shared a common T-cell phenotype CD3+CD8+, one patient was predominantly CD4+ and the other patient was dual negative for CD4 and CD8. Jovi-1 expression within the abnormal T-cell population of this group of patients was >90% restricted to one compartment; these findings were confirmed by TCR-Vβ-R analysis. Similar results were also obtained in each case of T-NHL and Sezary syndrome, more than 90% of T-cells from the population with an abnormal phenotype (CD3dim/CD4+CD8+, CD3+CD4+CD8+, CD3+CD4+CD8+, CD3+CD4+CD8+CD7− respectively) were either positive or negative for Jovi-1. Patients with persistent lymphocytosis were also assessed for Jovi-1 expression; in this group all patients had Jovi-1 positive and negative compartments within CD4 and CD8 T-cells.

Summary/Conclusions: In summary we have demonstrated a novel approach in the assessment of T cell clonality by targeting T-cell receptor β constant domain 1 (TRBC1). The addition of Jovi-1 in routine practice could improve the clinical evaluation of abnormal T-cell populations by flow cytometry.

E1141

A HIGHER AMOUNT OF LILOTOMAB PRE-DOSES INCREASING THE ACTIVITY-ADJUSTED AUC AND HAS A PROTECTIVE EFFECT AGAINST MYEOSUPPRESSION OF LUTETIUM (177Lu)-LILOTOMAB SATETRAXETAM IN RELAPSED INDOLENT NHL PATIENTS


1Radiolymphositatet, 2Oslo University Hospital, 3Nordic Nanovector, Oslo, Norway, 4Norrland University Hospital, Umeå, Sweden, 5University of Manchester, Manchester, United Kingdom

Background: lutetium (177Lu)-lilotomab satetraexetam (Betalutin®) is a novel CD37-binding murine IgG1 antibody radionuclide conjugate (ARC), in a ready-to-use formulation currently in Phase 1/2 clinical development for the treatment of non-Hodgkin lymphoma (NHL). Previously, pharmacokinetic (PK) data have been reported from 2 treatment arms of the ongoing LYMYRIT-37-01 study. In this abstract PK data from 4 treatment arms are presented for the first time.

Aims: This PK sub-study in iNHL patients (pts) was designed to determine the PK profile of 177Lu-lilotomab when administered after four different pre-dosing schedules.

Methods: Patients with relapsed incurable indolent NHL, with platelet counts ≥150 x109/L and <25% bone marrow involvement were eligible for inclusion in the study. All pts received either one or two doses of rituximab to deplete normal lymphocytes and all normal T-cells. A feature of the TCR is that the β-constant region comprises 2 functionally identical genes: TRBC1 and TRBC2. Each T-cell expresses only one of these. Consequently, normal T-cells will be a mixture of individual cells expressing either TRBC1 or 2, while a clonal T-cell disorders will exclusively express TRBC1 or 2.

Methods: Using multiparameter flow cytometry we assessed the expression of Jovi-1 in normal donors (n=19), T-cell leukaemia cell line (n=1), T-GL (n=9), T-NHL (n=3), Sezary syndrome (n=3) and patients with reactive lymphocytosis (n=4). A comparison of Jovi-1 and T-GL-Vβ-R was also performed to compare the two approaches.

Results: Jovi-1 expression within the CD4 and CD8 compartments of T-cells in normal donors was a median of 42.6% (range 33.7%-49%) and 36.4% (range 22.3%-48.5%) respectively. The T-cell line, Jurkat was exclusively positive for Jovi-1. Of the 9 patients with T-LGL, 7 patients shared a common T-cell phenotype CD3+CD4+CD8+, one patient was predominantly CD4+ and the other patient was dual negative for CD4 and CD8. Jovi-1 expression within the abnormal T-cell population of this group of patients was >90% restricted to one compartment; these findings were confirmed by TCR-Vβ-R analysis. Similar results were also obtained in each case of T-NHL and Sezary syndrome, more than 90% of T-cells from the population with an abnormal phenotype (CD3dim/CD4+CD8+, CD3+CD4+CD8+, CD3+CD4+CD8+CD7− respectively) were either positive or negative for Jovi-1. Patients with persistent lymphocytosis were also assessed for Jovi-1 expression; in this group all patients had Jovi-1 positive and negative compartments within CD4 and CD8 T-cells.

Summary/Conclusions: In summary we have demonstrated a novel approach in the assessment of T cell clonality by targeting T-cell receptor β constant domain 1 (TRBC1). The addition of Jovi-1 in routine practice could improve the clinical evaluation of abnormal T-cell populations by flow cytometry.
As compared to OFA alone, there was a decrease of 14% in Cmax and 15% in comparable when administered alone or co-administered with BEN (Table 1). Prior NHL therapy. PK concentration profiles and PK parameters of OFA were between treatment arms; the majority of patients from both arms did not receive included for PK parameters. Patient and disease characteristics were similar between treatment groups. The primary PK parameters Cmax, AUClast, AUCinf were derived using non-compartmental analysis. All adverse events (AEs) and severe AEs (SAEs) were recorded for safety assessments. Results: Thirty-two patients were randomized (15 in Arm A and 17 in Arm B), 3 patients in Arm A discontinued study treatment due to consent withdrawal (2 patients) and infusion related AE (1 patient). All 32 patients were included for safety and PK concentration analysis while 30 patients (15 in each arm) were included for PK parameters. Patient and disease characteristics were similar between treatment arms: the majority of patients from both arms did not receive prior NHL therapy. PK concentration profiles and PK parameters of OFA were comparable when administered alone or co-administered with BEN (Table 1). As compared to OFA alone, there was a decrease of 14% in Cmax and 15% in AUClast when OFA was co-administered with BEN, which was not considered relevant (Table 1). BEN PK concentration profiles and PK parameters were comparable with or without OFA co-administration (Table 1). All patients reported AEs. The most frequent treatment-related AEs were infusion related reaction in 53% and 47%, nausea in 33% and 35%, fatigue in 33% and 18% patients in Arm A and Arm B, respectively. The percentages of patients with grade 3/4 AEs were higher in Arm A (53%) compared to Arm B (24%). Cytopenias were present in 40% of patients in Arm A and 6% in Arm B. Four SAEs were related to study treatment in Arm A while none in Arm B.

Summary/Conclusions: A higher pre-dose of ililotomab increases the activity-adjusted AUC and decreases the volume of distribution and clearance rate of 177Lu-ililotomab in iNHL pts. Despite the increase in AUC the percentage reductions in neutrophil and platelet counts were smaller, indicating that a higher dose of ililotomab may have a protective effect against the myelosuppression associated with 177Lu-ililotomab. Further characterisation of 20 MBq/kg dose of 177Lu-ililotomab with 100mg/m² of ililotomab pre-dosing is ongoing and will be presented.

Table 1. Geometric mean (95% CI), Median (Min-Max) and Geometric Least Square Mean

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OPA- alone</th>
<th>OPA + BEN</th>
<th>OPA + BEN</th>
<th>OPA + BEN</th>
<th>OPA + BEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity adjusted Cmax (mg/l)</td>
<td>0.13</td>
<td>0.13</td>
<td>0.12</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Activity adjusted Cmax (mg/l)</td>
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<td>38</td>
<td>38</td>
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<td>Median (Min-Max)</td>
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<td>0.12 (0.08-0.18)</td>
<td>0.12 (0.08-0.18)</td>
<td>0.12 (0.08-0.18)</td>
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</table>

Summary/Conclusions: No relevant drug-drug interaction between OFA and BEN was observed in this study. OFA alone or in combination with BEN exhibited manageable safety profile in patients with iNHL.
Infectious diseases, supportive care

E1143

ASSESSMENT OF INTERNATIONAL CONSENSUS GROUP FOR HEMATOLOGY (ICGH) SMEAR REVIEW RULES FOR AUTOMATED PLATFORMS IN THE DETECTION OF MALARIA

J. Yan1,2,*, J.-D. Hu3, H. Huang4, M. Jiang5, J. Li6, M. Hou7, Y. Hu8, P. Wu9, J.-D. Hu10, S. Havyari11, T. Kato12
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Background: Peripheral blood smear review (SR) is a useful adjunct to the full blood count (FBC) and differential white cell count (DWCC), but is labor intensive and time consuming. For this reason, the international consensus group for hematology (ICGH) published guidelines to reduce SR rates in clinical laboratories using rules based on a combination of blood parameters and instrument suspect flags. These rules have reduced SR rates in many laboratories, but adjustment is often required to accommodate for local pathology/clinician preferences. As malaria is common in Johannesburg (JHB) (although not endemic), this study was undertaken to retrospectively evaluate the performance of modified ICGH SR rules for detection of malaria at the Chris Hani Baragwanath Academic Hospital Laboratory (CHBAH) (part of the National Health Laboratory Service (NHLHS) network) in JHB, South Africa.

Aims: To assess the performance of the CHBAH NHLHS SR rules in the detection of malaria.

Methods: Malaria test results (P. falciparum antigen & thick/thin SR) were extracted from the laboratory information system and corresponding FBCs assessed in those with parasitemia. All ICGH rules were applied to patients with both an FBC and DWCC requested, while only the parameter related rules were applied to patients with only a FBC performed. Samples were processed using a Sysmex XE-5000 analyser (Sysmex, Kobe, Japan), and the rules violated for each FBC recorded. As a retrospective laboratory based study, informed consent was not obtained. However, the study was approved by the Human Research Ethics Committee of the University of the Witwatersrand.

Results: Of the 153 samples included, all had P. falciparum parasitemia and 37 were collected from patients with severe malaria. A FBC with a DWCC was performed in 72/153(47.1%) patients, and a FBC alone in 81/153(52.9%). SR rules were triggered in 132(86.3%) patients (68(84.0%) in those with only a FBC performed, and 64(85.0%) in those with a FBC and DWCC). The thrombocytopenia (platelets (Ptl) <100x10^9/l) and anemia (Hb <7g/dl) rules were the most common, triggering in 105(79.5%) and 24(15.7%) patients respectively. Common analyzer morphology flags included those querying the presence of atypical lymphocytes, immature granulocytes and blasts, but 1/4 of these triggered in the absence of DWCC requested, while only the parameter related rules were applied to patients with only a FBC performed. Samples were processed using a Sysmex XE-5000 analyser (Sysmex, Kobe, Japan), and the rules violated for each FBC recorded. As a retrospective laboratory based study, informed consent was not obtained. However, the study was approved by the Human Research Ethics Committee of the University of the Witwatersrand.

Summary/Conclusions: SR rules are FN in 13.7% of patients with malaria, but are largely in those with near-normal blood counts. Furthermore, SR failed to identify the parasites in a further 13.0% of cases (particularly those with very low parasitemia). Elimination of a proportion of FN samples is thus not likely to be possible, and clinical vigilance for this condition is required. Reassuringly, SR rules were triggered in all the patients with severe malaria, and the parasites identified in 90.5% of these.

E1144

APPROPRIATE MULTICENTER STUDY OF CANDIDEMIA IN NEUTROPENIC PATIENTS WITH HEMATOLOGICAL DISEASES: INCIDENCE, RISK FACTOR AND OUTCOMES

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Background: Candidemia is one of the most common nosocomial bloodstream infections and is associated with morbidity and mortality, especially amongst the immunocompromised population. Several articles focus on the epidemiology of candidemia, but most of them were from cancer patients, patients with hematological malignancies, patients receiving solid organ transplant, or patients receiving hematopoietic stem cell transplantation (HSCT). Only 3 retrospective studies from single center described the clinical and microbiological features of candidemia in neutropenic patients with hematological malignancies.

Aims: A prospective, multicenter, observational study was designed to investigate the epidemiology, risk factors and outcomes of candidemia in neutropenic patients with hematological diseases.

Methods: This study was conducted in 11 hematological centers in China over a five-month period. From October 20, 2014 to March 20, 2015, consecutive patients of any age were included in this prospective study if they met the following criteria: (1) had hematological disease (2) experienced at least one episode of neutropenia during hospitalization.

Results: A total of 1139 consecutive cases were enrolled in this study. Out of 1139 neutropenic cases, 8 developed candidemia. The median time from neutropenia to diagnosis of candidemia was 18 days (range: 9-20 days). Among the cases, 3 patients were males, 5 females, 125 years old and 1 less than 18 years old. Six patients had acute myeloid leukemia (AML) and receiving induction chemotherapy. The cumulative incidence of candidemia in patients with AML and receiving induction chemotherapy was also significantly higher than that in patients receiving HSCT and other patients (5.45% vs. 3.19% vs. 0.00%, P=0.023).

Methods: Cases were stratified into low-risk group (0-2 risk factors), intermediate-risk group (2-3 risk factors), and high-risk group (4 risk factors). The cumulative incidence of candidemia was higher in high-risk group than that in intermediate-risk group and low-risk group (100.00% vs. 25.84% vs. 0.26%, P<0.001).

Summary/Conclusions: This study provided a description for the epidemiological study of candidemia in neutropenic patients with hematological diseases. This study defined the risk factors associated with candidemia in these patients, and confirmed that based on the risk factors, risk-stratification could identify the patients with a high-risk of candidemia.

E1145

BRONCHIALVEOLAR LAVAGE AS SYSTEMATIC APPROACH FOR EARLY DIAGNOSIS OF LUNG INFILTRATES AND INVASIVE PULMONARY INFECTIONS IN HEMATOLOGIC PATIENTS: A PROSPECTIVE SINGLE INSTITUTION STUDY

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Background: The best diagnostic approach of lung infiltrates (LI) remains to be established. Despite bronchoscopy with bronchoalveolar lavage (BAL) appears to be useful for LI diagnosis, hematologists and thoracic surgeons often have misdiagnosed in peripheral eosinophilic pneumonia at high-risk of procedure-related complications. A proper diagnostic approach at LI seems to be particularly relevant in neutropenic patients and/or in patients with an unfavorable clinical response to broad-spectrum antibiotics, in which the cause of LI are often filamentous fungi, as Aspergillus spp. To date, there were stratified, randomized controlled trials to apply in hematologic patients undergoing bronchoscopy for LI.

Aims: To evaluate the feasibility of bronchoscopy with BAL as systematic diagnostic approach at LI in hematologic patients, focusing on its role to diagnose invasive pulmonary aspergillosis (IPA).

Methods: Bronchoscopy was performed in all hospitalized patient with diagnosis of acute leukemia and LI at onset of disease before therapy start, and in any other hematologic patient in any phase of disease with LI requiring hospitalization because of concomitant febrile neutropenia and/or respiratory distress not responding to broad-spectrum antibiotics. Criteria for not response to broad-spectrum antibiotics were defined as: persistent (>48 h) fever, purulent respiratory secretions or purulent atrophy. In all cases we performed the same diagnostic work-up including blood-swabs cultures, serum galactomannan (GM) assessment (in three consecutive checks), serum beta-D-glucan, serum PCR for CMV, BAL...
fluid was studied by bacterial and fungal cultures, GM and PCR for Streptococcus pneumoniae, Legionella pneumophila, Chlamydia pneumoniae, Mycoplasma pneumoniae, Bordetella pertussis, Bordetella parapertussis, Haemophilus influenzae, respiratory virus including CMV, Pneumocystis jiroveci, Mycobacterium tuberculosis complex, Nocardia spp., Lysteria monocytogenes and Aspergillus spp. Available commercial kits were used according to manufacturer’s instructions.

Results: Out of 769 patients consecutively admitted in our ward, 85 had LI and 47 of them underwent BAL (total amount: 51 procedures). A causal agent of LI was detected in 33 cases (65%) allowing to modify the ongoing anti-microbial treatment in 25 of these ones (76%). Twelve cases of probable IPA, according to standard criteria, were diagnosed. Seven cases of LI fulfilling the radiologic criteria for IPA, though presenting only a positive Aspergillus PCR on BAL, were detected and treated as probable IPA. One life-threatening post-procedure complication was observed.

Summary/Conclusions: BAL seems a safe approach for an early diagnosis of LI in hematologic patients. The assessment of a broad diagnostic panel allowed the detection of a putative agent in 65% of cases. Assessment of Aspergillus by PCR on BAL proved useful for probable IPA diagnosis.

E1146

ESCAPE DRUG-RESISTANT INFECTIONS IN HEMATOLOGICAL MALIGNANCIES. DARE TO REVIEW!

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1Houston Methodist Cancer Center, 2Houston Methodist Research Institute, 3Houston Methodist Department of Hematology, Houston Methodist Hospital, HOUSTON, United States

Background: Patients with hematological cancers are at a high risk for increasingly resistant and severe infections. The Infectious Diseases Society of America has defined commonly resistant bacteria as ESKAPE (Enterococcus, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter, Pseudomonas aeruginosa, Enterobacter). As suggested in recent literature, other common and difficult-to-treat infections such as Clostridium difficile and Enterobacteriaceae organisms (E. coli, Proteus) can be added to this group and change the acronym from ESKAPE to ESCAPE.

Aims: We performed a retrospective review of the rate of ESCAPE infections, resistance profile, and outcomes in patients with various hematological malignancies at the Houston Methodist Hospital from 2006 to 2015.

Methods: The patient data was obtained from METEOR (Methodist Environment for Translational Enhancement and Outcomes Research), a clinical data warehouse that contains records dating back to January 1, 2006, with over 3 million patients and over 10 million unique patient encounters. We queried for leukemia (AML, CML, ALL, CLL), amyloidosis and myelodysplastic syndrome (MDS) along with hospitalizations due to bacterial infections. Baseline demographics and overall outcomes were also obtained.

Table 1.

Results: Out of 6017 patients with Hematological Malignancies, 660 patients with 684 malignant diagnoses were found; 235 had MDS, 174 had AML, 105 had ALL and 144 had CML. 39 had ALL, and 10 had an unspecified hematological cancer. Of 1132 infectious events, 62% were ESCAPE infections. The bacteria most frequently isolated were Enterococcus (23.4%), Staphylococcus aureus (18.5%) and Pseudomonas (16.9%). Bacteremia was the most predominant type of infection (41.9%) followed by urinary tract infections (38.2%). Patients with MDS (39.6%) and AML (25.3%) were mainly affected. A prevalent resistance to levofloxacin was detected in gram positives and gram-negative organisms (29-54%). Pseudomonas, E. coli, Proteus and Klebsiella pneumoniae showed a significant resistance to broad-spectrum antibiotics and higher mortality rates. A significant resistance to levofloxacin, a prophylactic antibiotic, was also noted. New strategies for reducing ESCAPE in MDS and AML are required. Further statistical review of this data set will be presented at the EHA Meeting, Madrid 2017.
Background: Neutropenic sepsis remains a leading cause of morbidity and mortality in both haematology-oncology and general oncology patients on cytotoxic chemotherapy. The authors published a preliminary report showing a beta lactam monotherapy in suspected neutropenic sepsis, discouraging the use of aminoglycosides, in view of potential toxicities. Increasingly in clinical practice it becomes evident that our patient population is incredibly heterogeneous and with the emergence of multi drug resistant strains of micro-organisms, high-risk individuals need to be identified early and first line antimicrobial treatment regimens tailored according to patient factors alongside local antibiogram.

Aims: To retrospectively review appropriate antibiotic use, microbial identification and antibiotic sensitivities amongst adult cancer patients with neutropenic sepsis. To identify if any patient or disease characteristics are associated with resistant isolates that would suggest the upfront usage of aminoglycoside containing antibiotic treatment regimens.

Methods: A retrospective review of patients treated for neutropenic sepsis was conducted for the period between 1/4/2015 to 11/10/2016. Analysis of potential risk factors including primary disease, age, sex, treatment regimen, albumin, neutrophil and lymphocyte count to assess potential association with adverse outcomes.

Results: There were 116 episodes of neutropenic sepsis in 92 patients in this period. Of these, 61 were haematology-oncology patients and 31 general oncology. 42 of 76 positive cultures identified gram-negative organisms. 40 patients received single agent Tazocin and 71 patients (61.2%) received Tazocin and an aminoglycoside as first line antimicrobial treatment. Fourteen isolates demonstrated resistance, including 2 cases of stenotrophomonas maltophilia and 12 cases of enterobacteriaceae. 13 of the 14 resistant isolates were found in haematology-oncology patients. Nine of these cases were resistant to single agent Tazocin but sensitive to an aminoglycoside. The mean age of cases with resistant bacteria was 54.2 years. There was no significant difference in sex or degree of neutropenia/lymphopenia in the cases that contracted resistant bacterial strains compared to those that were culture negative. Of the 4 fatal cases with resistant bacteria, 3 had low albumin (mean 25.5g/L cf. mean of 34g/L in resistant bacteria cases surviving).

Summary/Conclusions: This retrospective analysis supports the use of combination antimicrobials up front as first line treatment in high-risk patients with neutropenic sepsis. The study has demonstrated that the patient cohort most at risk of developing drug resistant bacteria are patients with high-risk or relapsed haematology-oncological disorders like AML or high-grade lymphoma, requiring multiple cycles of intensive chemotherapy. Of the patients who isolate resistant bacteria, identifying low albumin early may be a potential marker for adverse outcome in terms of morbidity and mortality. Of interest only one oncology patient isolated a resistant strain of bacteria, furthermore only 25% of general oncology patients treated with neutropenic sepsis had positive cultures compared to 75-8% of haematology-oncology patients. When comparing these findings to UK NICE recommendations it is clear that first line use of Tazocin in general oncology patients may well suffice in initial treatment of neutropenic sepsis. However with haematology-oncology patients early or up front consideration for the additional usage of an aminoglycoside is recommended to optimize outcomes in this high-risk population. From this study, the proposed risk factors of isolating resistant strains of bacteria leading to adverse outcomes would be aggressive haematological malignancies, receiving more intensive cytotoxic therapy, multiple lines of treatment and low albumin. Further analysis in a multi centre setting of the patient population would facilitate close collaboration between clinicians and microbiologists is essential in providing optimal anticoarbul therapy algorithms in neutropenic patients.

E1149 PRELIMINARY RESULTS FROM A LONG-TERM REPEAT DOSE TOXICITY AND TOXICOGENIC STUDY OF ANF-RHO, A NOVEL ANTI-NEUTROPENIC FACTOR, OR SAFETY PROFILE OF NEUTRAPROPHYLAXIS WITH ANF-RHO
J. Valenti1, J. Misra1, J.A. Newmark2
1Prolong Pharmaceuticals, Prolong Pharmaceuticals, South Plainfield, 2Toxicology, Toxikon, Bedford, United States

Background: ANF-Rho is a novel polyethylene glycol-modified granulocycol colony stimulating factor that has biological and biological properties that produce a prolonged pharmacokinetic and pharmacodynamic profile as compared to pegfilgrastim (Neulasta®). As such, it has potential applications in chemotherapy induced neutropenia and chronic idiopathic neutropenia. These disorders represent important unmet needs in neutrophil treatment and management of prophylaxis-induced neutropenia. The objective of this study was to investigate the safety profile of ANF-Rho administered in different treatment settings, to evaluate the long-term toxicity, genotoxicity and juvenile studies were conducted with ANF-Rho.

Aims: A 13-week study was conducted in Sprague Dawley rats and cynomolgus primates to assess various safety and pharmacokinetics of ANF-Rho as compared to Neulasta® (pegfilgrastim).

Methods: The study design used 288 rats, divided into 5 dosage groups: control, 100, 300, 1000 (high) and 1000 (positive) µg/kg. A total of 58 monkeys were also divided into 5 dosage groups: control, 75, 250, 750 (high dose) and 750 (positive) µg/kg of ANF-Rho. Doses were administered by weekly subcutaneous injections on Day 1, 8, 15, 22, 29, 36, 43 and 50. There was no difference in body weight and a dose dependent decrease in kidney weight in rats and a dose dependent difference in sex or degree of neutropenia/lymphopenia in the cases that contracted resistant bacterial strains compared to those that were culture negative. Of the 4 fatal cases with resistant bacteria, 3 had low albumin (mean 25.5g/L cf. mean of 34g/L in resistant bacteria cases surviving).

Summary/Conclusions: This retrospective analysis supports the use of combination antimicrobials up front as first line treatment in high-risk patients with neutropenic sepsis. The study has demonstrated that the patient cohort most at risk of developing drug resistant bacteria are patients with high-risk or relapsed haematology-oncological disorders like AML or high-grade lymphoma, requiring multiple cycles of intensive chemotherapy. Of the patients who isolate resistant bacteria, identifying low albumin early may be a potential marker for adverse outcome in terms of morbidity and mortality. Of interest only one oncology patient isolated a resistant strain of bacteria, furthermore only 25% of general oncology patients treated with neutropenic sepsis had positive cultures compared to 75-8% of haematology-oncology patients. When comparing these findings to UK NICE recommendations it is clear that first line use of Tazocin in general oncology patients may well suffice in initial treatment of neutropenic sepsis. However with haematology-oncology patients early or up front consideration for the additional usage of an aminoglycoside is recommended to optimize outcomes in this high-risk population. From this study, the proposed risk factors of isolating resistant strains of bacteria leading to adverse outcomes would be aggressive haematological malignancies, receiving more intensive cytotoxic therapy, multiple lines of treatment and low albumin. Further analysis in a multi centre setting of the patient population would facilitate close collaboration between clinicians and microbiologists is essential in providing optimal anticoarbul therapy algorithms in neutropenic patients.
Background: Voriconazole (VCZ) is a triazole antifungal agent with broad-spectrum activity against most clinically significant fungi. Its use in hematological patients is increasing, mostly in the form of intravenous formulation. The aim of this work was to report the prevalence of MPA isolates in a hematological ward and to determine the mortality rate after phase D.

Methods: We conducted a retrospective study from January 1991 to December 2015. Prospective study the cases of bacteremia and sepsis in 106 patients with hematological malignancies. Diagnostics of septic conditions was based on clinical data, bacteremia and systemic inflammatory reaction syndrome (SIRS) (registration of, at least, 2 of 4 clinical symptoms of SIRS). Bacteriological analyses and identification of microorganisms were performed by uniform technique over the entire study period, according to the valid guidelines. For DNA diagnostics, we used gene-specific PCR with real-time registration. DNA was extracted from peripheral blood leukocytes The herpetivirus panel included Herpes Simplex type 1 and 2 (HSV); Cytomegalovirus (CMV); Epstein-Barr virus (EBV), and Human Herpesvirus type 6 (HHV6). PCR techniques were performed according to manufacturer instructions.

Results: Based on the study 4923 blood samples it was shown that the frequency of detection of bacteria was 11.0%. The predominance of Gram-negative bacteria was demonstrated among pathogens detected in the bloodstream. However, the ratio of detectable Gram-negative flora was found to be increased from 23.1% to 39.6% between 2002 and 2015 (p<0.05). Coagulase-negative staphylococci (CoNS) prevailed among Gram-positive microorganisms, in particular, S. epidermidis, whereas Enterobacteriaceae, especially, E. coli, dominated among the Gram-negative bacteria. It is shown that the development of bacterial septicaemia was significantly more frequent on the background of the detection of Cytomegalovirus and the Epstein-Barr virus genomes. In recent years, this has increased the frequency of microorganisms detection in the blood of patients with hematological malignancies. In present study, antibiotic therapy started with β-lactame antibiotics combined with fluoroquinolones, aminoglycosides, metronidazole. If required, the antimicrobial strategy was revised 48-72 hours later as based on clinical and microbiological data, applying CYP2C19 no-wild-type. 2. 45 of 76 patients received voriconazole intravenous treatment of 3 out of 6 PA and 3 out of 4 MPF isolation sites. As a main corrective action, after 41 days we resumed admissions and approached phase D, resulting in a prompt and maintained decrease in isolates (Table 1).

Table 1. MPA isolates and mortality rate after phase D.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Number of Patients</th>
<th>Isolates</th>
<th>Mortality Rate</th>
</tr>
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<tbody>
<tr>
<td>Phase A</td>
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<td>45</td>
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<tr>
<td>Phase B</td>
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<tr>
<td>Phase C</td>
<td>51</td>
<td>52</td>
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</table>

Summary/Conclusions: We identified the contaminated water residue from BAW sink as a transmission route of MPA in our ward. Through measures of improving environmental measures, we were able to improve the efficiency of therapy and safety outcome.

E1153

BACTEREMIA AND SEPSIS FOLLOWING INTENSIVE CHEMOTHERAPY OF ADULT ONCOHematologic patients

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Background: Intensive cytostatic chemotherapy is a standard strategy for leukemia treatment. Meanwhile, such treatment causes negative effects, including lymphopenia, granulocytopenia and damage to tissue barriers associated with significant risks of infectious complications, especially, bacterial sepsis and viremia.

Aims: Our study was aimed for identification of bacteremia in oncohematological patients following intensive chemotherapy, and assessment of potential modifying role of herpesvirus infections.

Methods: Retrospective review of positive bacterial isolates of blood between January 1991- December 2015. Prospective study the cases of bacteremia and sepsis in cohort of 64 patients with hematological malignancies. Diagnostics of septic conditions was based on clinical data, bacteremia and systemic inflammatory reaction syndrome (SIRS) (registration of, at least, 2 of 4 clinical symptoms of SIRS). Bacteriological analyses and identification of microorganisms were performed by uniform technique over the entire study period, according to the valid guidelines. For DNA diagnostics, we used gene-specific PCR with real-time registration. DNA was extracted from peripheral blood leukocytes The herpesvirus panel included Herpes Simplex type 1 and 2 (HSV); Cytomegalovirus (CMV); Epstein-Barr virus (EBV), and Human Herpesvirus type 6 (HHV6). PCR techniques were performed according to manufacturer instructions.

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Summary/Conclusions: We identified the contaminated water residue from BAW sink as a transmission route of MPA in our ward. Through measures of improving environmental measures, we were able to improve the efficiency of therapy and safety outcome.
Iron metabolism, deficiency and overload

E1154
GLYCOSYLATED FERRITIN MEASURING SIGNIFICANCE FOR SECONDARY HEMOPOIETIC SYNDROME DIAGNOSTICS
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Background: Hemophagocytic syndrome (HPS) is a clinicopathologic condition characterized by systemic inflammatory reaction with cytopenia and tissue damage. The HFS may be primary (genetic associated) or secondary (SHPS), caused by different systemic disorders (immune, infectious, neoplastic). The overall clinical symptoms are similar to sepsis, so it could be difficult to differentiate among these entities. Ferritin levels are high in both cases, but the glycosylated/nonglycosylated ferritin fractions ratio is seems to be indicative.

Aims: The estimation of the ferritin fractions ratio and biochemical profile in patients with sepsis and SHPS.

Methods: The data from 64 patients were analyzed: 40 pts with diagnosed SHPS (median age 57, range 8-74 years) and 24 with lethal septic shock (median age 57.5, range 18-82 years). SHPS in patients with persistent fever refractory to antibacterial therapy and/or prolonged cytopenia and/or organ (lungs, CNS) involvement was established after the other conditions had been excluded. Sepsis diagnostics was based on the confirmed infection site and systemic inflammation with multiorgan failure. The following serum values were analyzed: alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), CRP, lactate dehydrogenase (LDH), bilirubin, creatinine, INR, C-reactive protein (CRP), procalcitonin (PCT), total ferritin, and glycosylated ferritin percentage. Mann-Whitney U test and ROC-analysis were used for statistical analyses.

Results: No differences were found in sepsis and SHPS for ALAT, ASAT, ALP, LDH, and bilirubin levels. The difference of INR, CRP, PCT, creatinine levels was significant (p<0.01). The most substantial difference in SHPS and sepsis groups had serum concentrations of ferritin, triglycerides, level of ferritin glycosylation (p<0.01) (Table 1). According to ROC-analysis, the area under the curve for ferritin, triglycerides and percentage of ferritin glycosylation were 0.78, 0.82, and 0.92, respectively.

Table 1.

Summary/Conclusions: The most difference between sepsis and SHPS was observed for triglycerides, ferritin and percentage of glycosylated ferritin. Percentage of glycosylated ferritin fraction seems to be the most indicative, which may make it useful for SHPS diagnostics and its differentiation from sepsis.

E1155
SERUM HEPcidIN QUANTIFICATION IN INFLAMMATORY BOWEL DISEASES
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1Dept. of Clinical laboratory and clinical immunology, 2Department of Propaedeutics of Internal diseases, 3Department of Medical chemistry and Biochemistry, Medical University, Sofia, Sofia, Bulgaria

Background: Inflammatory bowel diseases (IBD) include different intestinal pathologies, most common among them are Crohn Ulcerosa (CU) and Crohn’s Disease (CD). Pathogenesis of IBD is still unclear, however they are multifactor diseases, with genetic and autoimmune compounds, in combination of environmental factors. One of IBD symptoms is iron deficiency anemia.

Aims: We aimed to search for connection between serum hepcidin quantification and anemia in IBD.

Methods: We included 64 patients with IBD - 29 with Colitis Ucerosa (CU), and 35 with Crohn’s Disease (CD). They were diagnosed in University “Aleksandrovka” hospital in Clinic of Gastroenterology. Their results were compared to age and gender matched healthy controls. Laboratory assessments were analyzed for included groups – iron, ferritin, CRP, IL-6 and hepcidin. AAS, nephelometric, ELISA and statistical methods were used during analyzes and obtained results interpretation.

Results: 53 from our patients had with iron deficiency anemia (IDA) and low hepcidin concentrations (5.9±1.1 µg/L) compared to control group (19.9±2.8 µg/L). 11 of included cases had combination of IDA and hepcidin deficiency anemia (ACH). Their hepcidin levels were increased (59.9±6.4 µg/L) in comparison to healthy controls (19.9±2.8 µg/L); P<0.001. In patients with ACD/IDA, quantified serum hepcidin correlates positively to increased IL-6 (r=0.758, P<0.005) and CRP concentrations (r=0.899, P<0.001).

Summary/Conclusions: Quantification of serum hepcidin levels in IBD patients might be a key element in diagnosis and treatment of anemia in these patients. Serum hepcidin levels are useful marker for differential diagnosis between iron deficiency anemia and combination iron deficiency anemia/ anemia of chronic disease.
Results: Of these 11 patients, 7 were CDA type II. The median age of diagnosis was 12 months (3-144) months and male to female ratio was 7/4. Median transfusion requirement per year at previous year prior to initiation of chelation was 12 times (0-17). All of the patients were on chronic transfusion programme at initiation of iron chelation except for 2 (one receives occasional transfusion, and the other patient was on chronic transfusion programme but became transfusion independent after splenectomy). The median age at last visit was 70 months (32m-40 years). The median value of serum ferritin at initiation of iron chelators was 822 ng/ml. All of the patients were initiated deferasirox for iron chelation at a median dose of 24mg/kg/day (10-40) and the median chelation follow-up duration was 27 months (2-54 months). Three of the patients were evaluated with cardiac and hepatic T2* assessment prior to and by the end of 1 year of chelation and none of the patients were found to have cardiac iron loading at chelation initiation, whereas 2 had severe and 1 had moderate LIC values. In the subsequent assessment under chelation of these 3 patients all still had cardiac T2* values above 20 ms, whereas 1 had mild and 2 had moderate LIC values. Serum ferritin levels prior to initiation and by the end of 1 year of treatment were compared and the difference was found statistically insignificant.

Summary/Conclusions: Patients with CDA are at risk for iron loading and they need to be screened for the iron loading periodically. The prompt chelation in these patients prevent organ failure risks at long term including cardiac failure, cirrhosis and endocrinopathies.

E1158

ORAL IRON CHELATION FOR TREATMENT OF HEREDITARY HEMOCROMATOSIS IN CHILDREN

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Background: Hereditary hemochromatosis (HH) very rarely presents during childhood. The most common form of HH in children is Juvenile Hemochromatosis (JH), a rare genetic disorder inherited with an autosomal recessive manner, resulting from mutations in either the hemochromatosis (HJV) (type 2A) or the hepcidin (HAMP) gene (type 2B). Early diagnosis and closely monitoring of iron overload indexes, namely, serum ferritin levels, transferrin saturation and tissue iron measurement by magnetic resonance imaging (MRI) are essential in order to prevent permanent organ damage and potentially life threatening complications (cirrhosis, diabetes mellitus, cardiac dysfunction, and hypogonadism). Therapeutic intervention in children with HH may be problematic, as erythropoiesis is invasive and may not be well tolerated in young children. Iron chelation therapy can be implemented as an alternative treatment to erythropoiesis.

Aims: The scope of this study was to evaluate the use of an oral iron chelation therapy in young children with HH.

Methods: 3 children (2 females and 1 male) were diagnosed with HH at the aged of 4, 6 and 8 years old, respectively, based on increased ferritin and transferrin saturation levels and exclusion of other not-iron-overload related causes of hyperferritinemia. Genetic analysis were performed in all 3 patients and showed positive results in 2 of them, while on the 3rd no genetic changes could be identified. All patients were on pre-symptomatic stage of the disease and they were referred for evaluation after hyperferritinemia was discovered on a routine screening examination. Liver iron concentration (LIC) and cardiac iron concentration were evaluated by MRI (table 1). Iron chelation therapy with deferasirox (DFX) at low dose (of 10mg/kg/24h) was initiated, after evaluation was completed and permission from regulatory authorities obtained.

Table 1. Clinical characteristics of the patients.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Gender</th>
<th>Ferritin (μg/dl)</th>
<th>Transferrin saturation (%)</th>
<th>LIC (mg/g)</th>
<th>Cardiac T1* (msec)</th>
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<td>1</td>
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<td>1725</td>
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<td>637</td>
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<td>3</td>
<td>F</td>
<td>353</td>
<td>105</td>
<td>2.9</td>
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</table>

Results: All 3 patients responded promptly to therapy and showed decreased levels of ferritin, LIC and cardiac iron concentration. Gastrointestinal disturbances were noted in 1 patient, which resolved with H2-blockers and with changing the treatment to 5d/wk (patient 2). Mild increase in serum creatinine (>33% from baseline but within normal range for her age) was observed in patient 3, which resolved with temporary cessation of the chelation therapy.

Summary/Conclusions: Although the mechanism of neutrophil hypersegmentation in iron deficiency anaemia is not clear, it is thought that iron acts as a cofactor in folate metabolism and / or DNA synthesis in granulocytes. There are a limited number of studies dealing with NH associated with IDA in the literature. However most of these studies were observational and did not include controls or were not blinded. Our study is the first to demonstrate the association of NH with iron deficiency anaemia in adults in the absence of megaloblastic anemia.

E1159

NEUTROPHIL HYPERSEGMENTATION IN ADULTS WITH IRON DEFICIENCY: A CASE-CONTROL STUDY

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Background: Neutrophil hypersegmentation (NH) has been accepted as a hallmark of the macrocytic anemias associated with the deficiency of cobalamin or folate. However, there are a small number of reports stating that NH might accompany iron deficiency anaemia. The aim of the present study was to determine the association of NH with iron deficiency (with or without anaemia).

Aims: The aim of the present study was to determine the association of NH with iron deficiency (with or without anaemia) in adults and also to compare neutrophil segmentation status in anemia group before and after oral or parenteral iron treatment.

Methods: Fifty-six patients with iron deficiency and 20 age and sex matched controls were included in this prospective, single blind, case-control study between February-November 2016. Subjects were included if they were ≥ 18 years of age, and had normal serum vitamin B12 and folate levels, liver, thyroid and renal function tests. Pregnant women and patients with a history of blood transfusion within last 3 months and/or those with acute renal failure, anaemia of chronic disease, hypothyroidism, additional cytopenias and infection were excluded. Patients with iron deficiency were divided into 2 groups being with iron deficiency anaemia (IDA) and iron deficiency without anaemia (ID). Those with anaemia were further evaluated prior and after iron replacement. Results of the study groups were compared to age and sex matched healthy controls. Blinded peripheral blood smear slides were evaluated by a haematologist by counting 200 neutrophils. Hypersegmentation was defined as reported by Bain et al. Iron deficiency was diagnosed based on the findings of iron parameters including serum iron, total iron binding capacity, and ferritin. Anaemia was defined according to the WHO recommendation. Cohort characteristics were given in Table 1.

Table 1.

<table>
<thead>
<tr>
<th>Age (years)</th>
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Results: Hypersegmentation was detected in 25 individuals with iron deficiency (45%) and 1 healthy control (5%). It was significantly more frequent in the IDA group (48.8%) than in the ID group (30.7%) [p<0.001]. After iron treatment 3 IDA patients’ peripheral blood smear were evaluated and with normalization of iron parameters and hemoglobin, hypersegmentation was undetectable. The study is still ongoing and rest of the ID group are still on treatment, their peripheral blood smears are to be examined after iron treatment is over.

Figure 1. Summary/Conclusions: Although the mechanism of neutrophil hypersegmentation in iron deficiency anaemia is not clear, it is thought that iron acts as a cofactor in folate metabolism and / or DNA synthesis in granulocytes. There are a limited number of studies dealing with NH associated with IDA in the literature. However most of these studies were observational and did not include controls or were not blinded. Our study is the first to demonstrate the association of NH with iron deficiency anaemia in adults in the absence of megaloblastic anemia.
Background: Immunosuppression with mTOR inhibitors (sirolimus or everolimus) has been associated with development of microcytic anemia after solid organ transplantation. The prevalence reaches 27 to 57% in the case of kidney transplantation. This anemia has been attributed to hepcidin increase induced by the inhibition of mTOR protein.1,2

Aims: To evaluate the prevalence of microcytic anemia after allelogeneic hematopoietic stem cell transplantation in patients receiving mTOR inhibitors. Methods: 61 consecutive allelogeneic stem cell reduced intensity conditioning (alloRIC) recipients were analyzed. In all cases, a non-related donor was used. Baseline disease was: 23 acute leukemia (37.7%), 12 non-Hodgkin lymphomas (19.7%), 10 myelodysplastic syndromes (16.4%), 7 Hodgkin lymphomas 7 (11.4%), 4 multiple myelomas (6.5%), 3 chronic lymphocytic leukemia (4.9%), and 2 myelofibrosis (3.2%). All of them received Fludarabine-based conditioning treatment and the combination sirolimus (mTOR inhibitor)-taclidomus (calcinurin inhibitor) as GVHD prophylaxis. Drug doses were adjusted according to blood levels and renal function. Levels of Hb, MCV and iron parameters were also systematically evaluated after alloRIC. Microcytosis was considered when MCV was below 80 fl.

Results: At 6 months 56 out of 61 (92%) were alive. Anemia was observed in 30 (49%) of them, with only 8 cases (13.1%) showing Hb level below 100 g/l. Microcytic anemia was diagnosed in 2 of them (3.3%). One patient showed an iron deficiency anemia due to gastrointestinal bleeding (Hb 94 g/l, MCV 69 fl, serum ferritin 21 µg/l). However, the second one, a 61-year old male with an acute leukemia, had a microcytic anemia with iron parameter changes similar to those observed in kidney transplantation and associated with increased hepcidin, (see table). Anemia progressively improved with sirolimus tapering.

Table 1.

|---|

Summary/Conclusions: In contrast to kidney transplantation, microcytic anemia related to immunosuppression with mTOR inhibitors was seldom observed in alloRIC recipients. However, this association should be taken in account in this setting, as a rare cause of anemia. In case of microcytic anemia, the evaluation of iron parameters and hepcidin provides the diagnosis of this rare type of anemia.

E1162

ORAL IRON ELEVATES SERUM IRON AND CONSEQUENTLY CHANGES IRON DISTRIBUTION IN LIVER AND ERYTHROCYTES

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Background: For renal anemia patients, there are several therapeutic options including erythropoiesis-stimulating agents (ESAs), intravenous and oral iron supplemetations. In terms of iron absorption, ESAs were known to activate iron absorption via down-regulation of hepcidin, a key mediator of iron metabolism, and consequent up-regulation of duodenal iron transporters divalent metal transporter 1 (DMT1) and ferroportin (FPN). On the other hand, in previous study, intravenous iron was demonstrated to deactivate absorption system via hepcidin elevation. However, iron absorption under oral iron supplementation have not fully evaluated yet.

Aims: In this study, we investigated the activity of iron absorption under oral iron supplementation in mice as well as under intravenous iron supplemetation. In addition, we also analyzed iron distribution under intravenous and oral iron supplementation.

Methods: To load iron orally, a diet including 200 ppm of iron was used as control and a diet including approximately 5000 ppm of ferric citrate was used as iron-rich diet. 6-week-old male C57BL/6Ncr mice were divided into 3 groups; control group, intravenous iron (IV iron) group, and oral iron (Oral iron) group (n=5). Mice in IV iron group were fed a control diet from days 0 and intravenously administered 0.4mg/mouse of iron-dextran on days 9. Mice in Oral iron group were fed an iron-rich diet from days 0 and intravenously administered 0.4mg/mouse of dextran in mice as vehicle on days 9. Mice in Oral iron group were fed an iron-rich diet from days 0 and intravenously administered 0.4mg/mouse of dextran as vehicle on days 9. Mice in control group were fed a control diet from days 0 and intravenously administered 0.4mg/mouse of dextran on days 9. All mice were euthanized by exsanguination under anesthesia with isoflurane on days 14. For analyses of iron absorption, serum hepcidin and iron were measured and expression of duodenal DMT1 and FPN were evaluated immunohistochemically. For analyses of iron distribution, blue staining was used and hematocrit value and hemoglobin contents in each reticuloocyte and erythrocyte were measured.

Results: Serum hepcidin levels in IV and Oral iron groups were significantly higher compared with control group. However, serum iron levels were elevated only in oral iron group. In immunohistochemical analyses, expression levels of duodenal DMT1 were not detected in all groups and expression levels of duodenal FPN in IV and Oral iron groups were significantly lower than control group. As for iron distribution in liver, iron was accumulated in reticuloendothelial cells in IV iron group, on the other hand, in Oral iron group iron was accumulated in parenchyma. In hematological analyses, although red blood cell and reticulocyte count were not significantly different among groups, Ret-He and MCH in Oral iron group were higher than IV iron groups.

Summary/Conclusions: It was demonstrated in this study that serum iron levels were elevated in spite of high hepcidin levels and down-regulation of duodenal iron transporters under oral iron supplementation. Furthermore, iron was distributed in liver parenchyma and hemoglobin contents in each reticuloocyte and erythrocyte were up-regulated only under oral iron supplementation. We speculated that high serum iron lead to excess iron uptake into tissues and erythrocyte fraction. These data might provide an opportunity to rethink the importance of proper use of iron supplemetations.
Background: Children with haemoglobinopathy and rare anaemias often require regular red cell transfusions at some stage of their lives. Iron overload is therefore inevitable and iron chelation is a key component of therapy for children in this group. However its use has not been validated especially in children under two years of age. Deferasirox (Exjade®; Novartis Pharma AG, Basel, Switzerland) is an iron chelator that is conclusively proven to be effective and safe in transfusional anaemia such as haemoglobinopathies.

Aims: We aim to look at the efficacy and safety of Deferasirox in children with severe anaemias.

Methods: We present a case report of 6 children with severe anaemias treated with Deferasirox in a tertiary pediatric hematology centre in London, UK.

Results: Here we report 6 cases where Deferasirox has been used in young children with rare anaemias and sickle cell disease. Patients 1 and 2 presented within the first year of life with pancytopenia requiring regular transfusion and were diagnosed with Pearson syndrome. Deferasirox was started at the age of 8 months and was started on chronic transfusion program. Deferasirox was started at around the age of 1. He had a successful maternal haplo-identical haematopoietic stem cell transplant at the age of 3 years old. Transfusion and deferasirox were subsequently stopped.

Figure 1.

Summary/Conclusions: All of these children had stabilization or improvement of ferritin values after initiation of deferasirox as shown in figure 1. Deferasirox is licensed in Europe to be used in children with thalassaemia older than 6 years of age or older than 2 year of age when desferoxamine therapy is inappropriate or inadequate. Deferasirox is preferable in severe anemias due to iron overload such as celiac disease, gastrointestinal disorders and other chronic conditions. Local Physicians were free to prescribe any oral iron formulation, according to their standard practice. A calendar of laboratory test was suggested, including basal assessment of whole blood count, reticulocytes, iron status, and subsequent checkpoints at 3 days (WBC and reticulocytes only), 2 weeks, 6 months. Clinical data regarding compliance to therapy, unwanted effects, final outcome were recorded.

Results: 112 (M 58) patients were enrolled. Ethnic distribution was: Caucasian 74, African 23, Asian 10, Other 5. The median age at diagnosis of IDA was 1.5 years, with a bimodal distribution with frequency peaks at age 3 and 12-14 yrs-yls. Sixty-eight patients received bis-glycinate ferrous 0.45mg/kg, 19 elemental iron (ferrous gluconate/sulfate) 2mg/kg, 12 liposomal iron 0.7-1.4mg/kg, and 15 other preparations. Eating habits were reported as normal in 48 patients, inadequate weaning in 21, meat/fish restriction in 32, other in 11. Gastro-intestinal side effects were reported in 9/68 (13%) of the bis-glycinate iron group, in 3/19 (16%) of the elemental iron group, and in 0/12 of the liposomal iron group. Suspension of therapy due to side effects was needed only in 5 patients, 4 in the bis-glycinate and 1 in the elemental iron group, respectively. Final outcome was available for 77 patients; it was recorded as solved IDA, persistent IDA, or lost at follow up. Solved cases were 40/53 (75%) in the bis-glycinate iron group, 4/11 (36%) in the elemental iron group, and 8/13 (62%) in the liposomal iron group. Persistent cases were 8/53 (15%) in the bis-glycinate iron group, 6/11 (55%) in the elemental iron group, and 1/13 (8%) in the liposomal iron group. Lost at follow up were 5/53 (9%) in the bis-glycinate iron group, 1/11 (9%) in the elemental iron group and 4/13 (31%) in the liposomal iron group.

Summary/Conclusions: The collected data show that both bis-glycinate and liposomal iron formulations have a good efficacy/safety profile and offer a sustainable alternative to classic elemental iron preparations.

E1165

AN INVESTIGATION ABOUT WEIGHT GAIN WITH TREATMENT OF IRON DEFICIENCY ANEMIA: CHANGES OF GHIRELIN AND HEPcidIN LEVELS WITH TREATMENT

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Background: Iron deficiency anemia (IDA) is a global health problem and problems in compliance with oral iron therapy are frequently seen. It has been shown that medications are not used regularly or discontinued due to weight gain during the treatment process.

Aims: We investigated ghrelin, known as appetite hormone and its relationship with hepcidin, the homeostatic regulator of intestinal iron absorption, in order to explain some symptoms of IDA and weight gain during iron treatment.

Methods: A hundred and twenty adult IDA patients, referred to our clinic between October 2015 and October 2016 were included in the study. The study was completed with 87 patients, who gave the informed content and a control group consisted of 50 healthy people. Information about age, gender, weight, height, body mass index (BMI), waist-hip circumference and blood samples were taken from the patient and control groups. The treatment of IDA was done according to the dose and method recommended by the responsible physician, the researchers did not have any effect on the treatment. Measurements and blood tests were repeated in the patient group after normalization of the anemia parameters, not before the third month of treatment. Hepcidin and ghrelin levels
were examined once in the control group and twice in the patient group, before and after treatment.

Results: When the patient and control groups were compared, there was no significant difference in terms of age, sex, height, weight, BMI, waist and hip circumference. The pretreatment plasma hepcidin and ghrelin levels of the patient group were significantly lower than those of the control group (80±21 ng/ml vs 179 ng/ml p <0.001 for hepcidin, 152±119 pg/ml vs 213±167 for ghrelin, p=0.026). There was a significant increase in terms of weight (mean 1.15 kg, p <0.001), BMI (25.86 kg/m² vs 26.33 kg/m², p <0.001), waist and hip circumference measurements (mean 0.81cm in both, p <0.001) after treatment in the patient group. After treatment, the levels of hepcidin were significantly increased compared to the pre-treatment levels (80±21 ng/dl vs 92±13 ng/dl p <0.001). Although an increase in the plasma ghrelin levels was encountered after treatment, it was not statistically significant (152±119 pq/ml vs 164±150 pq/ml, p=0.589). When correlations of individual increases in ghrelin levels were examined, a weak positive correlation was found between increase in ghrelin levels and weight gain.

Summary/Conclusions: In our study, ghrelin was significantly lower than the control group in the IDA group, suggesting that it may be the cause of loss of appetite. Ghrelin is also detected in neurons of hypothalamic arcuat nucleus, which regulates appetite. The deficiency of iron may cause deficiencies in enzymatic activities of iron depended enzymes and it may disturb the function of these neurons. But the increase with treatment did not reach statistical significance. This may be due to physiological suppression of levels by weight gain. When patient-based weight gains were examined, there was a positive but weak correlation with the increase in ghrelin levels of those people. Hepcidin was significantly lower in the iron deficiency group than in the control group in the IDA group, suggesting that it may be the cause of loss of appetite. Ghrelin is also detected in neurons of hypothalamic arcuat nucleus, which regulates appetite. The deficiency of iron may cause deficiencies in enzymatic activities of iron depended enzymes and it may disturb the function of these neurons. But the increase with treatment did not reach statistical significance. This may be due to physiological suppression of levels by weight gain. When patient-based weight gains were examined, there was a positive but weak correlation with the increase in ghrelin levels of those people. Hepcidin was significantly lower in the iron deficiency group than in the control group in the IDA group, suggesting that it may be the cause of loss of appetite. Ghrelin is also detected in neurons of hypothalamic arcuat nucleus, which regulates appetite. The deficiency of iron may cause deficiencies in enzymatic activities of iron depended enzymes and it may disturb the function of these neurons. But the increase with treatment did not reach statistical significance. This may be due to physiological suppression of levels by weight gain. When patient-based weight gains were examined, there was a positive but weak correlation with the increase in ghrelin levels of those people. Hepcidin was significantly lower in the iron deficiency group than in the control group in the IDA group, suggesting that it may be the cause of loss of appetite. Ghrelin is also detected in neurons of hypothalamic arcuat nucleus, which regulates appetite. The deficiency of iron may cause deficiencies in enzymatic activities of iron depended enzymes and it may disturb the function of these neurons. But the increase with treatment did not reach statistical significance. This may be due to physiological suppression of levels by weight gain. When patient-based weight gains were examined, there was a positive but weak correlation with the increase in ghrelin levels of those people. Hepcidin was significantly lower in the iron deficiency group than in the control group in the IDA group, suggesting that it may be the cause of loss of appetite.

WHOLE GENOME MBD-SEQ REVEALS DIFFERENT CPG METHYLATION PATTERNS IN AZACYTIDINE-TREATED JUVENILE MYELOMONOCYTIC LEUKEMIA (JMML) PATIENTS

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Background: Juvenile Myelomonocytic Leukemia (JMML) is a rare and aggressive leukemia of early childhood. Allogeneic hematopoietic stem cell transplant (HSCT) is the only available curative treatment, but, since disease recurrence is responsible for treatment failure in at least one third of transplanted patients, developing alternative therapeutic approaches is desirable. Aberrant DNA methylation is a key molecular feature of JMML, suggesting an important role of epigenetic events in the pathophysiology of the disease. Azacytidine (AZA), a molecule that inhibits DNA methylation in human cells, is under investigation in JMML.

Aims: Here we report, for the first time, a global evaluation of DNA methylation status of CD34+ cells deriving from JMML patients before and after AZA treatment and compared the results with those of healthy controls. Identifying differentially methylated CpG islands linked to various genes will help us describe
an epigenetic aberrant paradigm possibly involving transcripational and translational regulation in JMML.

Methods: CD34+ cells isolated from 3 JMML patients samples collected at diagnosis (t0) and after the third cycle of AZA (t1) were evaluated together with those of 3 healthy donors (HD). JMML patients have been treated with AZA on a compassionate use basis after obtaining signed informed consent. DNA samples were processed and Ion fragment libraries were prepared. MBD-seq, bioinformatics and statistical analysis were performed by Genomnia srl (Bresso, Italy).

Results: First, we compared 10 JMML cells with HD cells, finding 987 different transcriptional units corresponding to 714 coding and 273 non-coding sequences. We also compared DNA methylation between t0 and t1, finding a hypermethylation both in pre- and post-AZA samples compared to HD, confirming a broad genomic hypermethylation both in pre- and post-AZA samples compared to HD. In this comparison, 644 unique transcriptional units, including 468 coding and 176 non-coding sequences, were found. Hypermethylation in JMML samples compared to HD was detected, but, unexpectedly, t0 vs t1 methylation analysis did not show any significant result, suggesting a likely unspecified patient-related pharmacological effect. Notably, 453 coding and 165 non-coding differentially methylated regions are shared between t0 vs HD and t1 vs HD sets. More in detail, 261 and 15 coding regions and 107 and 10 non-coding regions were uniquely found in t0 vs HD and t1 vs HD sets, respectively. However, 439 coding and 161 non-coding genomic regions preserve their hypermethylated status, probably due to a mechanism of resistance to AZA treatment. Among non-coding elements, we found different RNA species, such as microRNAs, splicing RNAs, lincRNAs/antisense transcripts (AS) and other unknown RNAs. Retrotransposons, belonging to LINEs and SINEs families, were also screened. We identified 13 sequences with a significant differential methylation profile in both t0 and t1 vs HD. Again, a comparison between t0 and t1 groups did not show any significant difference. Eleven hypermethylated common LINEs were evident between t0 vs HD and t1 vs HD sets. Two retrotransposons with opposite methylation patterns were found in t0 vs HD and t1 vs HD sets; while in the first comparison they included LINEs, in the second one they are 1 hypermethylated LINE and 1 hypomethylated SINE.

Figure 1.

Summary/Conclusions: In conclusion, the whole genome MBD-seq performed for the first time on JMML CD34+ bone marrow derived cells, showed a broad genomic hypermethylation both in pre- and post-AZA samples compared to HD, suggesting a patient-specific AZA-effect. Transcription and translation processes of coding and non-coding genes could be deregulated in multiple ways, due to heterogeneity of sequences involved in CpG islands hypermethylation. Moreover, due to their known ability to insert random mutations in the genome, retrotransposons should be considered as a candidate for further studies in JMML pathogenesis.

E1168

RESPONSE MONITORING IN MDS WITH DEL(5Q) USING DIFFERENT FLOW CYTOMETRIC (FCM)-SCORES IN COMPARISON TO CYTOGENETICS AN ELNET IMDS-FLOW EXPERIENCE

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Background: Flow cytometry (FCM) is one part of integrated MDS diagnostics. Different well established FCM-scores are applied, as FCSS (Wells et al. 2003), Ogata-score (Ogata et al. 2012), new iFS (Cremers et al. 2017), and del(5q)-FCM-score (Oelschlaegel et al. 2015).

Aims: The aim of this prospective study was to test, which of the mentioned FCM-scores fits best for response monitoring in del(5q) MDS in comparison to cytogenetics.

Methods: Overall, 245 FCM investigations were performed in 61 patients with MDS and del(5q) (IPSS-R very low/low: n=26, int: n=13, high/very high n=22) including 42 patients with isolated del(5q) or one additional cytogenetic abnormality. The majority of analyses were performed in patients receiving lenalidomide or azacitidine (n=29 and n=22 patients), or in patients receiving chemotherapy and/or allogeneic transplantation or growth factors (n=10). Standardized FCM (lyse-stain-wash) and cytogenetics/FISH procedures were performed according to ELN guidelines at the TU of Dresden, VUMC of Amsterdam, UH of Guadalajara and UH of Bristol. Cytogenetics/FISH analysis was considered the gold standard. All of the applied FCM-scores were propagated by the ELNet MDS working group. Additionally, hematological improvement of the erythroid lineage (Hi-E) was evaluated (Cheson et al. 2006).

Results: The del(5q)-FCM-score reflected best the disappearance / presence of the cytogenetic abnormality del(5q) with a sensitivity of 98% and a specificity of 82%. This was confirmed if only MDS with del(5q) as a single abnormality or only MDS treated with Lenalidomide were evaluated separately (sensitivity: 98% and 100%; specificity: 85% and 75%). The use of the Ogata-score considering almost only abnormalities of the myeloid progenitors, ended up with a slightly lower sensitivity (86%) and specificity (81%). The new iFS analyzing progenitor cells, granulo-, mono-, and erythropoiesis showed a comparably high sensitivity (83%) but a slightly impaired specificity (72%). FCSS, analyzing dyspoiesis of multiple cell lineages, showed a response in less than half of all investigations being in cytogenetic CR (sensitivity: 41%), but revealed a high specificity (91%). The analysis of Hi-E was high sensitive (81%) but not as specific (62%). Next, we investigated the potential prognostic impact of response monitoring using various FCM-scores compared to cytogenetics. Considering all del(5q) MDS patients as well as only those patients with del(5q) as a single abnormality, cytogenetics and all tested FCM-scores showed a significantly longer OS for MDS responding to therapy. The highest prognostic impact disappeared for the iFS (p=0.0019) and Ogata-score (p=0.0092), respectively. Evaluating only MDS treated with lenalidomide, response monitoring using FCSS separated best the OS curves (p=0.0080). Finally, we combined the evaluation of Hi-E with cytogenetics or the FCM-scores. This resulted in an even better CS for MDS fulfilling two response criteria vs none (p=0.0092) as the highest prognostic impact for the combination of Hi-E plus the new iFS (p=0.0010).

Summary/Conclusions: Flow cytometry might serve as a rapid tool for response monitoring during treatment with disease-modifying drugs. All established FCM-scores allowed for an at least similar correctness of response prediction. The prognostic impact of the various FCM-scores seems to be even higher than that of cytogenetic response evaluation in this MDS subgroup. One reason might be, that most of the FCM-scores reflect not only the genetic background of the MDS but dyspoietic alterations in various cell lineages of the hematopoietic system.

E1169

EVALUATION OF MUTATIONS AT RELAPSE IN MYELODYSPLASTIC SYNDROME PATIENTS RECEIVING ALLOGENIC STEM CELL TRANSPLANTATION

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Background: Allogeneic transplant (AlloSCT) is the only curative therapy for myelodysplastic syndromes (MDS). Unfortunately, relapse is the main cause of treatment failure. Evaluation of genetic mutations both at diagnosis and
before AlloSCT is a potent prognostic tool. However, mutational profile at relapse after AlloSCT has not been widely explored.

Aims: In this study, we evaluate mutational profile at post-AlloSCT relapse in MDS patients to determine if pre-AlloSCT mutations are still present at relapse, so we could eventually monitor them as minimal residual disease (MRD) after AlloSCT.

Methods: From a retrospective cohort of 115 patients, we selected those who relapsed post-AlloSCT (19/115, 16.5%) with available material at relapse (18 patients). We performed an in-house target-capture panel, sequencing across selected exons of 117 cancer-related genes previously related to MDS in pre-AlloSCT samples to identify genetic mutations and we checked the presence of those mutations in same at relapse. Six patients were discarded because lack of pre-AlloSCT mutations, so we selected 12 patients for the sequential study. DNA was amplified with FastStart High Fidelity PCR System using exon-specific primers for each mutation. The indexed paired-end library was prepared with Nextera XT DNA Sample Preparation Kit (Illumina) The median coverage per base was achieved was 4570 reads range (28-8573). In a second step, we explore the possibility of evaluating mutations in both CD34 positive and the rest of bone marrow cells, to check if we could increase the sensitivity of the detection.

Results: Median age of relapsed patients was 60 (45-70). Diagnosis were RAEB 1 (n=4), RAEB 2 (n=4), dysplasia associated AML (n=2) and RCDM (2). They relapse post-AlloSCT after a median of 2.5 months (1-7), and 4 of them are alive at last follow up after a median of 22 months (9-33). Patients had a median of 2.5 mutations (range 1-4), TET2 mutations were detected in 4 (33%) of patients; U2AF1, EZH2, SRSF2, KRAS, JAK2 and RUNX1 in 2 (17%), and NRAS, TP53, ETV6, PHF6, SMC1A, ZRSR2, BCR, DNTM3 and SF3B1 mutations in 1 (8%) (Table 1). In 10 out of 12 evaluated patients, we found same genetic mutations at relapse compared with pre-AlloSCT sample (Table 1). In addition, mutational pattern was similar for all patients except for one in which dominant mutation at relapse was SRSF2 present in 14% of cells pre-Allo and in 3% at relapse) instead of ETV6 (51% pre-AlloSCT and 0.6% at relase). In 2 patients, pre-AlloSCT mutations were not detected at relapse (Patient 8; BCR and RUNX1. Patient 11; SRSF2, TET2 and RUNX1). In a second step, we searched for mutations in CD34 positive cells to check its sensitivity to detect genetic alterations. We selected CD34 positive cells in one patient with KRAS and IDH2 mutations pre-AlloSCT. KRAS and IDH2 were present in 40% and 45% of CD34 positive cells and in 37% and 48% of the bone marrow (CD34 depleted) compartment respectively in pre-AlloSCT samples. In relapse samples, mutations were present in similar percentage in CD34 positive cells compared to CD34 depleted bone marrow (KRAS 0.83% and 2.23%; IDH2 1.6% and 1.45% respectively).

Table 1. Mutations before and after the AlloSCT in relapsed patients

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Summary/Conclusions: Post-AlloSCT relapsing MDS show same genetic mutations found in pre-AlloSCT evaluation, so they would potentially be used to confirm clonality and probably MRD assessment after AlloSCT in the near future. CD34 selection does not provide additional sensitivity to whole bone marrow cellularity sample.

E1170
RIGOSERTIB COMBINED WITH AZACITIDINE EPIDEMIOGENICALLY MODULATES CHROMATIN AND HEMATOPOIETIC STEM CELL POPULATIONS IN THE MYS

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Background: Azacitidine (AZA) is the standard of care for patients (pts) with higher-risk MDS, however, only 50% of pts respond and the majority will relapse within 2 years. All pts ultimately fail treatment due to primary or secondary resistance. Rigosertib (RIG) is a “ras mimic” agent that binds to the Ras Binding Domain of RAF kinases and inhibits the RAS-RAF-MEK and the PI3K pathways. Initial results of an ongoing Phase III study with RIG combined with AZA showed significant benefit with MDS del5q. To further explore the effects of RIG combined with AZA in pts with del5q, we analyzed the expression of hematopoietic stem and progenitor cell markers by flow cytometry in response to RIG/AZA treatment in the Bone Marrow (BM) of del5q patients with MDS.

Methods: We analyzed 26 patients with MDS del5q (11 at the time of the CRF and 15 at the time of the CRF after RIG/AZA treatment). BM samples were analyzed for CD34+ hematopoietic stem and progenitor cells (HSPC) with markers for pluripotency, differentiation, self-renewal, and telomere lengths. HSPC were analyzed before and after RIG/AZA treatment.

Results: CD34+ hematopoietic stem and progenitor cells (HSPC) (ANOVA, p=0.006) and the RIG/VOR induced 1.9-fold expansion (H3K4me3 and H3K4me2) and acetylation (H3K9ac, & H3K18ac) levels associated with transcriptional activation or repression in both the cell lines and pt samples. Q-PCR studies demonstrated that individual treatment of BW90 and MDS-L with RIG or combined with AZA and VOR in sequential treatment (AZA/RIG, RIG/AZA, VOR/RIG or RIG/VOR) altered DNA methyl transferases (DNMT1, 3a and 3b), the class I, II and IV histone deacetylases (HDACs), and chromatin remodeler (KDM2a, SET1, JMJD3 and LRWD1) transcript levels in a cell line specific context. Sequential treatment of RIG with AZA or VOR demonstrated differential effects on the association of Pol II (H3K4me3) with the associated genes (Pol II) (51% pre-AlloSCT and 0.6% at relapse post-AlloSCT after a median of 2.5 months (1-7), and 4 of them are alive at last follow up after a median of 22 months (9-33). Patients had a median of 2.5 mutations (range 1-4), TET2 mutations were detected in 4 (33%) of patients; U2AF1, EZH2, SRSF2, KRAS, JAK2 and RUNX1 in 2 (17%), and NRAS, TP53, ETV6, PHF6, SMC1A, ZRSR2, BCR, DNTM3 and SF3B1 mutations in 1 (8%) (Table 1). In 10 out of 12 evaluated patients, we found same genetic mutations at relapse compared with pre-AlloSCT sample (Table 1). In addition, mutational pattern was similar for all patients except for one in which dominant mutation at relapse was SRSF2 present in 14% of cells pre-Allo and in 3% at relapse) instead of ETV6 (51% pre-AlloSCT and 0.6% at relase). In 2 patients, pre-AlloSCT mutations were not detected at relapse (Patient 8; BCR and RUNX1. Patient 11; SRSF2, TET2 and RUNX1). In a second step, we searched for mutations in CD34 positive cells to check its sensitivity to detect genetic alterations. We selected CD34 positive cells in one patient with KRAS and IDH2 mutations pre-AlloSCT. KRAS and IDH2 were present in 40% and 45% of CD34 positive cells and in 37% and 48% of the bone marrow (CD34 depleted) compartment respectively in pre-AlloSCT samples. In relapse samples, mutations were present in similar percentage in CD34 positive cells compared to CD34 depleted bone marrow (KRAS 0.83% and 2.23%; IDH2 1.6% and 1.45% respectively).

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Summary/Conclusions: Post-AlloSCT relapsing MDS show same genetic mutations found in pre-AlloSCT evaluation, so they would potentially be used to confirm clonality and probably MRD assessment after AlloSCT in the near future. CD34 selection does not provide additional sensitivity to whole bone marrow cellularity sample.

E1171
UNEXPLAINED CYTOPENIAS IN HOSPITAL: INDICATIONS AND BENEFITS OF NEXT-GENERATION SEQUENCING

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Background: Unexplained cytopenias (UC) are common problems during hospitalisation, particularly in elderly patients. If there is no evident cause, myelodysplastic syndrom (MDS) is frequently suggested and a bone marrow aspiration is performed. Next-generation sequencing (NGS) reveals MDS-asso- ciated genetic mutations but these are not always diagnostic. In our centre, NGS was systematically realized in the context of unexplained cytopenias.

Aims: The objective of this study was to explore results of NGS in practical routine in the context of UC and to precise if some groups of patients could more specifically benefit from NGS.

Methods: All patients in our centre with analysis of NGS performed in blood or in bone marrow in a context of UF were included. Exclusion criteria were: patients under 18 years, monocytes ×1000/mm³, excess of blasts, history of hematological malignancy disorder. Patients were included in group “positive NGS” if at least one significant mutation (no SNP) was found on 25 genes and in group “no NGS” if no significant mutation was found.

Results: Of 480 UC, NGS was systematically realized in the context of unexplained cytopenias. The objective of this study was to explore results of NGS in practical routine in the context of UC and to precise if some groups of patients could more specifically benefit from NGS. In our centre, NGS was systematically realized in the context of unexplained cytopenias. The objective of this study was to explore results of NGS in practical routine in the context of UC and to precise if some groups of patients could more specifically benefit from NGS. In our centre, NGS was systematically realized in the context of unexplained cytopenias. The objective of this study was to explore results of NGS in practical routine in the context of UC and to precise if some groups of patients could more specifically benefit from NGS. In our centre, NGS was systematically realized in the context of unexplained cytopenias.
Results: 156 patients were included between January 2014 and December 2015 with a mean age of 68 years [65.8-70.3] and 47.4% of men. 127 patients (81.4%) had a bone marrow analysis. 53 patients (34.0%) were reported in the group “positive NGS” and 103 patients (66.0%) in the group “negative NGS”. In univariate analysis, significant variable associated with “positive NGS” were age (p<10-7), no history of auto-immune disease (p=0.002), hemoglobin <12g/dl (p=0.017), platelets >150000/mm³ (p=0.018), >10% dysplastic cells in erythroid (p=0.012) and granulocytic lineage (p=0.034). Trend test on dysplastic lineage number was significant (p=0.006). Normal karyotype (78.1%) was comparable in the two groups (p=0.352). Cirrhosis and/or portal hypertension were comparable in the two groups (14.1%, p=0.092) as well as mean serum creatinine (p=0.24). In multivariate analysis, age >70 years (p=0.0015) and platelets >150000/mm³ (p=0.0213) remained significantly associated to positive NGS (Table 1). In “positive NGS” group, 1 (58.5%), 2 (32.1%), 3 (7.5%) or 4 (1.9%) mutation(s) were found per patient. Most frequent mutations were TET2 (25.9%), DNMT3A (17.3%), SF3B1 (12.3%), ASXL1 (12.3%), SRSF2 (8.6%), U2AF1 (4.9%), TP53 (3.7%) and ZRSR2 (3.7%). Other mutations were reported in less than 3 patients. As expected in this elderly population, if a unique mutation was found, TET2 and DNMT3A were predominant (35.5% and 25.8% respectively) but interestingly mutation R862 of DNMT3A was found in only one patient. Sideroblasts were found in 15% in 46.2% of patients with a mutation of SF3B1, SRSF2, U2AF1 or ZRSR2.

Table 1.

<table>
<thead>
<tr>
<th>Total Positive NGS</th>
<th>Total negative NGS</th>
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<tbody>
<tr>
<td>39</td>
<td>117</td>
</tr>
<tr>
<td>28</td>
<td>89</td>
</tr>
<tr>
<td>19</td>
<td>78</td>
</tr>
<tr>
<td>10</td>
<td>48</td>
</tr>
<tr>
<td>0</td>
<td>57</td>
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</table>

Summary/Conclusions: In the context of unexplained cytopenias, a third of patients had at least one MDS-associated somatic mutation. Age above 70 years and no thrombopenia seems to be good arguments to realize NGS in this context. Probably thrombopenia is frequently associated to other causes than MDS. Positive NGS is positive, aging genes are the most frequently mutated genes and they can reflect age-related clonal cytopenia. Even if their clinical significance is uncertain, monitoring is recommended because of an increased risk of hematologic cancer.

E1172

Abstract withdrawn.

E1173

RESISTANCE TO AZACITIDINE IS DETERMINED AT CELLULAR LEVEL BY LOWER EXPRESSION OF NUCLEOSIDE ACTIVATING ENZYMES UCK1 AND UCK2

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Background: Azacitidine is at present the standard treatment for MDS. We demonstrated that MDS patients responsive to azacitidine have significantly higher intracellular expression of the azacitidine-activating enzyme uridine-cytidine kinase-1 (UCK1) in bone marrow mononuclear cells (Valencia et al. Leukemia 2014). Correlation of the expression of nucleoside transporter, activating and deactivating enzymes with clinical outcomes of azacitidine and decitabine has been suggested by several authors. Yet, the crucial role of these enzymes has to be ascertained, as well as their possible different importance in determining resistance to azacitidine.

Aims: To confirm that the cellular expression of nucleoside metabolizing enzymes plays a major role in cellular resistance and significantly impacts on clinical response to azacitidine.

Methods: Two cell lines, SKM1 sensitive (SKM1-S) and SKM1 resistant (SKM1-R) to azacitidine, were analyzed for expression of UCK1, UCK2, hENT1, hCNT3, RRMI and RRMM by quantitative PCR. Corresponding proteins were observed in Western blotting of cell lines. One of the azacitidine resistant cell line SKM1-R and UCK2 was blunted by siRNAs in SKM1 sensitive cells to determine their role in in vitro sensitivity to azacitidine. For UCK1 and UCK2 silencing in SKM1-S, specific siRNAs were used (OnGene Technologies, MD, USA); cells were cultured at a density of 600x10⁶ cells/ml in 5 ml of RPMI 1940 medium. After 72 h of transfection, cells were treated for further 48h with azacitidine at the concentrations of 0.1 and 1 μM. After assessment of effective gene silencing, apoptosis and cell cycle arrest were evaluated, respectively by Annexin V test and Propidium Iodide. In parallel, the percentage of 5-methylcytosine was quantitated by ELISA assay (Global DNA Methylation LINE-1 kit ActeMotif, CA, USA). In conclusion, the expression of nucleoside metabolizing enzymes was evaluated prospectively in 18 IPSS high risk MDS patients treated with azacitidine 75mg/m²/7 days every 28 days. Furthermore, UCK1 and UCK2 expression was evaluated in 37 patients (classified as 26 responder and 29 non-responder) treated with azacitidine, by RNAseq analysis using DEXSeq. Results: SKM1-R cells did not express UCK1, UCK2, RRMI and RRMM. Corresponding proteins were also not expressed. A reduction of apoptosis was observed in UCK1-silenced SKM-1 S after azacitidine 0.1 μM treatment: 35.7±3.07% Annexine V-positive cells versus 25±3.03% (P=0.031) in non-silenced control SKM1-S cultures. We observed a reduction of apoptosis due to UCK2-silencing after azacitidine 0.1 μM treatment too: 31.0±3.85% Annexin V-positive cells versus 21±3.05% (P=0.054). Hypomethylation induced by in vitro azacitidine treatment was also hampered by reduction of expression of UCK1 and UCK2. Quite surprisingly gene expression of UCK1, UCK2, hENT1, hCNT3, RRMI and RRMM in primary cells did not predict different clinical response to azacitidine. RNAseq analysis for UCK1 and UCK2 did not find any differences between responder and non-responder patients.

Summary/Conclusions: We demonstrated that UCK1, UCK2, hENT1, hCNT3, RRMI and RRMM and the corresponding proteins are absent in azacitidine-resistant cell line SKM1-R suggesting to be the determinant of the induced resistance to azacitidine. Reduced expression of UCK1 and UCK2 significantly decreased azacitidine effects. Prospective evaluation of the predictive role of cellular expression of genes involved in azacitidine metabolism is ongoing in a larger cohort of MDS patients.

E1174

FAMILIAL TIN2 N-TERMINAL LOSS OF FUNCTION MUTATION IN TELOMERE SYNDROME

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Background: The shelterin complex protects telomeres from being processed by the DNA damage repair machinery and regulates telomere access and structural integrity (Frank 2015). About thirty mutations are known in Telomere Syndrome proteins, i.e. TRF1, TRF2 and TPP1, thus contributing to telomere length regulation and structural integrity (Frank 2015). About thirty TINF2 mutations are known in Dyskeratosis Congenita (DC) (Savage 2008) and other telomere related phenotypes, i.e. aplastic anemia (AA), idiopathic pulmonary fibrosis, liver cirrhosis, myelodysplastic syndromes (MDS) and acute myeloid leukemia (Armanios 2012). All mutations were missense and heterozygous, clustering in exon 6 encoding for a highly conserved segment of the C-terminus (aa 280-291) (Frank 2015).

Aims: Precise diagnosis in AA/MDS with clinical features of telomere syndrome. Methods: AA was diagnosed in a 69-year old man, with a multisystem disorder, i.e. pсорiasis, nail dystrophy, severe osteoporosis, chronic hepatitis, mild chronic kidney failure and hypertension, suggesting a telomere syndrome. Karyotype was normal. Patient was unresponsive to immune-suppressive therapy. DNA from peripheral blood and hair bulbs was analyzed for TERT, TERC and telomere status by southern blotting in both cell lines. The expression of TINF2, TRF1, TRF2 and TPP1, thus contributing to telomere length regulation and structural integrity (Frank 2015). About thirty TINF2 mutations are known in Dyskeratosis Congenita (DC) (Savage 2008) and other telomere related phenotypes, i.e. aplastic anemia (AA), idiopathic pulmonary fibrosis, liver cirrhosis, myelodysplastic syndromes (MDS) and acute myeloid leukemia (Armanios 2012). All mutations were missense and heterozygous, clustering in exon 6 encoding for a highly conserved segment of the C-terminus (aa 280-291) (Frank 2015).

Results: SKM1-R cells did not express UCK1, UCK2, RRMI and RRMM. Corresponding proteins are also not expressed. A reduction of apoptosis was observed in UCK1-silenced SKM-1 S after azacitidine 0.1 μM treatment: 35.7±3.07% Annexine V-positive cells versus 25±3.03% (P=0.031) in non-silenced control SKM1-S cultures. We observed a reduction of apoptosis due to UCK2-silencing after azacitidine 0.1 μM treatment too: 31.0±3.85% Annexin V-positive cells versus 21±3.05% (P=0.054). Hypomethylation induced by in vitro azacitidine treatment was also hampered by reduction of expression of UCK1 and UCK2. Quite surprisingly gene expression of UCK1, UCK2, hENT1, hCNT3, RRMI and RRMM in primary cells did not predict different clinical response to azacitidine. RNAseq analysis for UCK1 and UCK2 did not find any differences between responder and non-responder patients.

Summary/Conclusions: We demonstrated that UCK1, UCK2, hENT1, hCNT3, RRMI and RRMM and the corresponding proteins are absent in azacitidine-resistant cell line SKM1-R suggesting to be the determinant of the induced resistance to azacitidine. Reduced expression of UCK1 and UCK2 significantly decreased azacitidine effects. Prospective evaluation of the predictive role of cellular expression of genes involved in azacitidine metabolism is ongoing in a larger cohort of MDS patients.
Summary/Conclusions: A new TIN2F germinal variation at exon 2, c.254A>G p.H85S, was identified in the proband and in both brothers. Screening on 200 healthy donors was negative. Significantly short telomeres were found in proband (p=0.0161) and brothers (p=0.0082 and p<0.0001), compared to age and sex matched controls. The proband had a normal SNPs profile and WES identified an additional somatic variation in TLR1 gene (c.1859G>A, p.R620Q). Co-immunoprecipitation experiments showed that the new TIN2F mutation reduced TIN2 binding with TRF2 in vitro.

E1175
FUNCTIONAL EXPRESSION OF TIM-3 AND CLINICAL SIGNIFICANCE OF PLASMA GALEN-9 LEVELS IN MYELODYSPLASTIC SYNDROMES
T. Asayama1, M. Ishibashi1, H. Tamura1, Y. Kurabayashi-Hamada1, N. Takada-Ouyama1, A. Onodera-Kondo1, K. Moriya1, N. Yokose2, K. Inokuchi1
1Dorns (n=9) using ELISA, 2Department of medicine, Nippon Medical School, Tokyo, 2Division of hematology, Department of internal medicine, Chiba Hokuso Hospital, Chiba, Japan

Background: T-cell immunoglobulin and mucin domain-3 (Tim-3) is an inhibitory immune checkpoint molecule that suppresses adaptive immunity by binding with galectin-9 (gal-9). The Tim-3–gal-9 pathway is associated with self-renewal of leukemic stem cells in acute myeloid leukemia (AML), although the function of the axis in myelodysplastic syndromes (MDS) remains unclear.

Aims: To clarify the expression and function of Tim-3 and clinical impact of the ligand gal-9 in MDS.

Methods: 1) We evaluated Tim-3 expression on CD45-gating blasts of bone marrow mononuclear cells (BMMCs) in 20 patients with MDS and AML transformed from MDS (AL-MDS), 12 healthy controls, and 4 MDS cell lines using flow cytometry (FCM). 2) To investigate Tim-3 induction, MDS cell line F-36P cells were co-cultured with the culture supernatant of human stromal cells and flow cytometry (FCM). 3) To eludicate the functions of Tim-3 on MDS cells, F-36P cells were divided into Tim-3– and Tim-3+ fractions with FACS sorting and their differential gene expression was determined with oligonucleotide microarray analysis. 4) To investigate the proliferative potential of Tim-3 signaling, intracellular Ki-67 expression in F-36P cells was evaluated using FCM when co-cultured with/without anti-Tim-3 blocking antibody. 5) Finally, we analyzed gal-9 concentrations in culture supernatants of MDS cells and in plasma obtained from patients with MDS (n=51) and AL-MDS (n=19), and healthy donors (n=10).

Results: 1) Tim-3 expression was observed on monocytes and CD45-gating blasts in MDS BMMCs in all 4 MDS cell lines. In AL-MDS patients, Tim-3 expression levels on blasts were markedly higher than in controls and MDS patients (p<0.0001). 2) Tim-3 induction may be essential for cell proliferation associated with the culture supernatant of human stromal cells and the MDS-related cytokine transforming growth factor-β (TGF-β). Tim-3 cell-surface protein and mRNA expression in MDS cell lines was induced by co-culture with TGF-β. The Tim-3 induction was abrogated by adding the TGF-β receptor I kinase inhibitor SD208. 3) Microarray analysis showed 572 upregulated genes (>2-fold difference) and 304 downregulated genes (<0.5-fold difference) in Tim-3–F-36P cells compared with Tim-3+ cells, and ingenuity pathway analysis of those genes revealed upregulation associated with cell proliferation and antiapoptotic responses in Tim-3+ cells. 4) The blockade by anti-Tim-3 antibody decreased intracellular Ki-67 expression in F-36P cells, suggesting that Tim-3 signaling induced MDS cell proliferation. 5) Soluble gal-9 was detected in culture supernatants of MDS cell lines and PBMCs obtained from AL-MDS patients. Soluble gal-9 levels and gal-9 mRNA expression were upregulated by MDS-related cytokines interferon-γ and tumor necrosis factor-α. Plasma gal-9 levels were higher in MDS and AL-MDS patients than in healthy controls (P=0.0001). When MDS patients were divided into high (defined as >10 ng/mL) and low (<10 ng/mL) gal-9 groups, the high group had poorer overall survival compared with the low group (P=0.001), even in refractory anemia (RA)/RA with ringed sideroblasts patients (P=0.0029). Multivariate analysis revealed that a high gal-9 level was an independent poor prognostic factor (P=0.0017).

Summary/Conclusions: Our data suggest that Tim-3 expression and plasma gal-9 levels were upregulated in advanced-stage MDS. Tim-3 is associated with cell proliferation of MDS blasts, and higher plasma gal-9 is a poor prognostic marker in MDS. These molecules could play a key role in MDS disease progression.

E1176
PROGNOSTIC SIGNIFICANCE OF GENE MUTATIONS IN MDS DEPENDS ON THE LOCUS OF GENE VARIANCES
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Background: Myelodysplastic syndromes are a collection of clonal hematopoietic disorders with a wide range of clinical manifestations and eventual outcomes. Predicting the prognosis is of great importance for defining the risk and choice of treatment options. A number of risk stratification tools exist, all of which include genetic markers along with other clinical and paraclinical features. The Revised International Prognostic Scoring System (IPSS-R, Greenberg et al., Blood. 2012;120(12): 2454-2465) defines 5 risk levels based on the presence of specific chromosome abnormalities. These genome aberrations provide evidence for disease although reports of frequent driver mutations (Papamichael et al., Blood, 2013) and/or structural variants detected by single nucleotide polymorphism (SNP) arrays (Tiu et al., Blood, 2011) have shown a potential for score criteria in the diagnosis of MDS. Recent reports of the presence such genetic aberrations in disease free individuals makes this approach problematic (Genovese et al., N Engl J Med 2014; Lichman, Blood 2015, Kwok et al., Blood 2015). A study of patients without evidence for MDS identified a driver mutation and/or structural gene variants in 91% of pre-diagnostic samples with the mutational spectrum mirroring that seen in MDS population (Cogo et al. Blood, 2015). The presence of mutations with greater median variant allele fraction (40% vs 9% to 10% in healthy individuals) and occurring with additional mutations (>2 mutations, 64% vs 8%) were shown to define a high-risk group with a shorter time to disease progression and poorer overall survival.

Aims: To compare the genomic profile of bone marrow from 145 adults, 76 of whom met the WHO criteria for MDS.

Methods: All samples were screened by chromosome G banding or molecular karyotyping using 8x60K oligonucleotide arrays (Agilent, USA) or screened by FISH using probes (Cytocell, UK) targeting the most common aberrations associated with MDS as per IPSS-R classification (Greenberg et al., Blood, 2013). The commercially available target gene panel TrueSight on a MiSeq platform (Illumina, USA) was used to screen mutational hotspot in 5 cancer related genes relevant to myeloid malignancy. Gene variances were reported at allele frequencies (VAF) >10% and at minimum read depth of 300 as per manufacturers criteria. We used the Catalogue of Somatic Mutations In Cancer (COSMIC), dbSNP and 1000 genome (>2%) to classify gene variants as either drivers, variants of unknown significance and germline polymorphisms (SNPs).

Results: A total of 145 bone marrow samples from 58 women and 87 men, aged from 26 to 85 suspected to have myeloid dysplasia were investigated. Of these only 76 (52%) were found to fulfill the WHO, criteria referred to as MDS positive, the rest as MDS negative. Gene variances were detected in all but 7 samples. The latter group was devoid of gene mutations. We observed driver mutations as reported in myeloid malignancies in 68 (47%) samples whilst 70 (48%) were found to carry the same variances seen in disease free individuals or of unknown significance. As expected driver variances were not identified in any of the samples that failed the WHO criteria for MDS. Variances were detected in all samples for 35 of the 54 genes targeted by the TrueSight panel. In order of frequency these are TET2, SRSF2, ASXL1, CUX1, DNMT3A, RUNXI, BCOR1 and HRAS, seen in more than 10% of samples, while the rest were less frequently reported. The aberrant genes ASXL1, TET2 and SRSF2 figured prominently in both groups of samples with comparable frequencies, although they were absent in 37.3% of disease free samples. In disease free samples, a total of 73 variances were observed, 70 (48%) were found to carry the same variances seen in disease free individuals or of unknown significance. As expected driver variances were not identified in any of the samples that failed the WHO criteria for MDS. Variances were detected in all samples for 35 of the 54 genes targeted by the TrueSight panel. In order of frequency these are TET2, SRSF2, ASXL1, CUX1, DNMT3A, RUNXI, BCOR1 and HRAS, seen in more than 10% of samples, while the rest were less frequently reported. The aberrant genes ASXL1, TET2 and SRSF2 figured prominently in both groups of samples with comparable frequencies, although they were absent in 37.3% of disease free samples. In disease free samples, a total of 73 variances were observed, 70 (48%) were found to carry the same variances seen in disease free individuals or of unknown significance.

Summary/Conclusions: We compared 145 bone marrow samples from patients presenting with MDS of which 76 met the WHO criteria. There is little difference in their genomic profile when comparing the two groups on the basis of the most highly involved genes (ASXL1, TET2 and SRSF2) but we compare the two groups by variance, 9 variances are exclusively associated with MDS positive disease.
Methods: Besides azacitidine and decitabine, three other agents (SGI-1027, zebularine, and gemcitabine) are known as having hypomethylating effect. In vitro activities of the 5 HMA's on HMA resistant cell lines (MOLM/AZA-1 and MOLM/DEC-5) were tested by cell viability assay using luminescent-based CellTiter-Glo system. Protein and mRNA levels of DNMT enzymes (1, 3A, and 3B) were assayed before and after treatment of each HMA. Proteosomal degradation and activation of Akt were also determined to see the correlation with changes of DNMT's.

Results: Although azacitidine and decitabine could suppress DNMT1 and DNMT3A in MOLM-13, the agents could not suppress DNMT enzymes in resistant cell lines. Inhibition of proteosomal degradation by bortezomib induced accumulation of DNMT enzymes in MOLM-13, whereas it did not accumulate the enzymes in MOLM/AZA-1 and MOLM/DEC-5. Phosphorylated Akt (p-Akt) was dramatically overexpressed in MOLM/AZA-1 and MOLM/DEC-5. SGI-1027 showed the lowest IC50 values for MOLM/AZA-1 and MOLM/DEC-5, and it suppressed the protein levels of all three DNMT enzymes. SGI-1027 could also decrease the level of p-Akt. GDC-0941, a PI3K inhibitor, suppressed DNMT1 and DNMT3A as well as p-Akt, but it could not decrease DNMT3B in MOLM/AZA-1 and MOLM/DEC-5. Cell viability assay showed the synergistic effects of combination of GDC-0941 and Nanamycin A, a specific DNMT3B inhibitor, in MOLM/AZA-1 and MOLM/DEC-5.

Summary/Conclusions: DNMT levels of MOLM/AZA-1 and MOLM/DEC-5 were not dependent on proteosomal degradation. DNMT1 and DNMT3A might be regulated via PI3K-Akt pathway, while regulation of DNMT3B might be different from DNMT1 and DNMT3A. SGI-1027 appears to exert inhibitory effects on MOLM/AZA-1 and MOLM/DEC-5 by inhibition of both p-Akt and DNMT3B.

E1178

MECHANISTIC HIGHLIGHTS OF IMPROVED ERYTHROPOIESIS WITH A LOW DOSE OF DEFERASIROX IN LOW RISK MYELODYSPLASTIC SYNDROMES

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Background: Myelodysplastic syndromes (MDS) are a group of heterogeneous clonal stem cell disorders leading to ineffective hematopoiesis. Anemia is a frequent cytopenia in MDS and the majority of patients requires red blood cell (RBC) transfusion resulting in the development of iron overload (IO). Deferasirox (DFX) became a standard treatment of IO in MDS and seems to have positive effects on hematopoiesis with a reduced need of RBC transfusion.

Aims: Decipher the mechanisms of the potential improvement of erythropoiesis with DFX.

Methods: We report our in vitro data about the proliferation, cell cycle, apoptosis, erythroid differentiation, and cell signaling pathways concerning CD34+ hematopoietic stem progenitor cells from low risk MDS samples in a 2-step erythroid differentiation liquid culture with low dose DFX and iron overload.

Results: We observed a higher proliferation rate for cultures with 3µM DFX versus the control condition (p=0.038). In contrast, no increased proliferation was found with DFX>5µM and with other chelators used in the clinic. The higher proliferation rate with DFX 3µM was due to the combination of decreased apoptotic cells at day 10 (D10) (p=0.03) and D14 (p=0.007) and increased cycling cells at D10 (p=0.0001). Regarding clonogenic assays, there were more CFU-E colonies with DFX 3µM (p=0.04). Despite the low concentration of DFX, cells exposed to DFX 3µM had a lower intracellular iron concentration measured by ICP-MS than control cells (p=0.019). Nevertheless, this decreased iron amount was not sufficient to activate cellular iron regulation by Iron Regulatory Proteins suggesting the absence of a direct effect of low dose DFX on iron homeostasis. Moreover, low dose DFX decreased intracellular and mitochondrial reactive oxygen species (ROS) at D14 (p=0.048 and p=0.03) and decreased the level of malonaldehyde (p=0.048), a product of lipid peroxidation. Then, we have investigated which signaling pathways were sensitive to DFX 3µM. We found an increased nuclear translocation of NFκB detected by both CM (p=0.04) and luciferase reporter assay (p=0.03). NFκB activation was absent in the knock-down (KD) condition. Moreover, in non-iron overloaded medium condition, the level of ROS was not increased, and DFX in the TRX1 KD condition was not associated with NFκB activation. These results suggest that NFκB activation in this model is linked to TRX1 and regulated by an extremely fine control of ROS levels with a likely threshold effect.

Summary/Conclusions: Our study describes the pro-proliferative effects of low dose of DFX on erythroid progenitors in low risk MDS patients. These results provide a biological rationale for a clinical trial which will propose low dose of DFX in MDS patients, refractory to erythropoiesis stimulating agents.
Myelodysplastic syndromes - Clinical

**E1179**  
**EVALUATING ERYTHROBLAST PAS POSITIVITY IN THE DIAGNOSTIC APPROACH OF MYELODYSPLASIS SYNDROME**  
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**Background:** According to WHO minimal morphological criteria for myelodysplastic syndrome (MDS) diagnosis, at least 10% of bone marrow (BM) cells of at least one hematopoietic lineage must show unequivocal dysplasia to be considered as dysplastic. Morphological abnormalities of erythroid cells include cytoplasmic Periodic acid-Schiff (PAS) positivity, but the diagnostic power of this cytochemical reaction is not yet fully clear.

**Aims:** The aims of our study were to evaluate the diagnostic significance of erythroblast PAS positivity in MDS and to investigate a possible correlation between levels of PAS positivity and other morphological and clinical features.

**Methods:** We retrospectively examined the results of the cytochemical PAS staining for glycogen in BM smears from 165 patients with MDS, 116 patients with non-clonal cytopenia and 49 healthy subjects. We developed a PAS score by counting 100 nucleated cells for the erythroid lineage and classifying them according to the degree of PAS reactivity. The discriminant power of both PAS positivity rate and score for MDS identification was evaluated in comparison with that of the conventional morphological features of dyserythropoiesis; then, PAS positivity was included into the morphological scoring system we have previously defined (Leukemia 2015;29:66-75).

**Results:** PAS positive erythroblasts were observed in 104 (63%) MDS patients, 46 (40%) patients with non-clonal cytopenia, and 12 (24%) non-erythroid nucleated cells, with a significant difference between MDS and non cytopenic controls (p<0.0001) or non-clonal cytopenias (p=0.0001), but not between healthy controls and non-clonal cytopenias (p=0.09). In MDS, both positivity rates (median 2%, range 0-33) and scores (median 2, range 0-33) were significantly higher than those in normal and pathological controls (p=0.0001 and p=0.0004 for rate, p=0.0001 and p=0.0002 for score, respectively), without significant difference in relation to excess blasts or multilineage dysplasia. MDS patients with >4% ring sideroblasts (RS) showed lower PAS positivity rates and scores than MDS patients with <4% RS (p=0.0332 and p=0.0412, respectively). In MDS, erythroblast PAS positivity was not influenced by SF3B1 mutation status. In MDS, no significant relationship was detected between erythroblast PAS positivity and percentage of BM blasts, percentage of BM erythroblasts, dyserythropoiesis grading, or Hb levels, whereas an inverse correlation was noticed between PAS score values and internal bridging (r=-0.23, p=0.0395). A ROC curve analysis allowed us to identify a PAS score value ≥1 (AUC=0.674, p=0.0034) as optimal cutoff to discriminate MDS patients from non-clonal cytopenias. Considering the most discriminant morphological features for dyserythropoiesis, the weight of both PAS positivity rate and score in the identification of BM dysplasia was lower than that of ring sideroblasts and megablasts, but higher than that of defective hemoglobinisation, nuclear lobulation, multinuclearity, cytoplasmic fraying, pyknosis, and internuclear bridging. Introducing conventional parameters and PAS results significantly improved the sensitivity of our morphological scoring system.

**Summary/Conclusions:** The evaluation of BM erythroblast PAS positivity, easy to perform and inexpensive, may be useful in the work-up of patients with suggested MDS, especially if there is only unilineal dysplasia without ring sideroblasts or excess blasts.

**E1180**  
**A PHASE 3 RANDOMIZED PLACEBO (PBO)-CONTROLLED DOUBLE-BLIND TRIAL OF DARBEPOETIN ALFA IN LOW OR INTERMEDIATE-1 (INT-1) RISK MYELODYSPLASIS SYNDROMES (MDS)**  
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**Background:** There is a lack of PBO-controlled data for erythropoiesis-stimulating agents (ESAs) in MDS.

**Methods:** To evaluate darbepoetin alfa (DAR) in IPSS low/int-1 risk MDS (Randomized controlled trial E 2000-016522-14, NCT01362140).

**Aims:** Patients with MDS per WHO 2008 criteria with IPSS low/int-1 risk, anemia [hemoglobin (Hb)<10 g/dL], low transfusion burden, no previous treatment with ESAs or biologic response modifiers, and serum EPO≤500mU/mL were randomized 2:1 to 24 weeks (wk) SC DAR 500 µg or PBO every 3 wk (Q3W), stratified by IPSS, then 48 wk open label (OL) DAR. Follow-up is ongoing. Doses were withheld for Hb>12g/dL and decreased if Hb increased by >1.5 g/dL in 3 wk. Key endpoints were transfusion incidence and Hi-E per IWG 2006.

**Results:** Randomized patients [N=147] had median Hb of 9.3 (min-max:5.5-10.6) g/dL and median baseline EPO of 69 (min-max:4.3-497) mU/mL. WHO classification was RA:15%, RARS:14%, RCMD:44%, del5q:9%, RAEB-1:16%, and MDS-U:unknown:2%. Transfusion incidence wk 5-24 was significantly reduced with DAR [DAR:36.1% vs PBO:59.2%, p=0.008]. In the 48-wk OL DAR period, 50.8% of patients had transfusions. More DAR patients achieved Hi-E in the double blind period [DAR:14.7% (11/75 evaluable) vs PBO:0% (0/35 evaluable), p=0.016]. In the 48-wk OL DAR period, 34.7% (34/98) of patients achieved Hi-E. Improved Hi-E and transfusion responses were seen with more favorable status for IPSS-R but not IPSS. In the 48-wk OL DAR period, dose frequency increased from Q3W to Q2W in 81% of patients; doses were held/reduced frequently. Safety results from this trial were consistent with the previous DAR phase 2 MDS trial, with similar AML rates in PBO and DAR arms.

**Figure 1.**

**Summary/Conclusions:** In this phase 3, randomized, double-blind, PBO-controlled trial in anemic IPSS low/int-1 risk MDS patients, 24 wk of darbepoetin alfa Q3W significantly reduced transfusions and increased Hi-E rates with no new safety signals. Most patients met criteria to change to Q2W dosing during the 48-wk OL period, suggesting that Q2W dosing may offer more benefit. The true clinical benefit of darbepoetin alfa may have been underestimated due to the nature of IWG 2006 Hi-E criteria and trial design (Hb measured Q3W, dosing rules).

**E1181**  
**PRELIMINARY ANALYSIS OF EFFICACY AND SAFETY OF SINTRA-REV CLINICAL TRIAL, LENALIDOMIDE VS PLACEBO PHASE 3 STUDY IN LOW/INT-1 MDS PATIENTS WITH DEL(5Q) AND TRANSFUSION INDEPENDENCY**  
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Background: Lenalidomide (LEN) is the first choice of treatment in low risk MDS patients with isolated del(5q) (MDS-del(5q)) and transfusion dependency (TD). Most of the low risk MDS-del(5q) patients diagnosed with anaemia and independent of transfusions developed TD or needed treatment for symptomatic anaemia early after diagnosis (median of 20 months, abstract 3180 ASH, 2016). LEN directly targets the del(5q) clone improving anaemia, quality of life and survival in these subset of patients. For these reasons, the use of LEN in patients with del(5q), anaemia and not TD seems to be very attractive. However, data about the use of LEN in MDS 5q- patients and transfusion independency (TI) are scanty, some retrospective studies suggest a benefit with the early use of LEN in this setting, but there is not already available any prospective and randomised study to confirm this likely advantage.

Aims: Our aims were to analyze efficacy and safety at week 12 of treatment with LEN vs Placebo in this setting of low risk MDS del(5q) patients with anaemia and not in TD at diagnosis

Methods: From 2010 to 2017, 47 patients have been included in the Sinstra-Revl trial, a phase III, multicenter, randomized and double blind study with LEN (5mg/day) vs placebo [2:1 randomization] in Low – Int-1 risk (IPSS) MDS del(5q) patients with anaemia but TI. Preliminary results of efficacy (according to the IWG 2006 criteria for erythroid [Hi-ER] and cytogenetic response [CyR]) and safety has been analyzed at week 12. Progression disease (DP) in the trial was defined as the development of TD.

Table 1.

<table>
<thead>
<tr>
<th>Age median (range)</th>
<th>Gender M/F</th>
<th>Hb (g/dL) median (range)</th>
<th>Neutrophils median (range)</th>
<th>Neutrophils in BM median (range)</th>
<th>IPSS-R (% in BM)</th>
<th>Low-very low</th>
<th>Low-low</th>
<th>Low-normal</th>
<th>Low-high</th>
<th>Low-very high</th>
<th>Partial</th>
<th>Complete</th>
</tr>
</thead>
<tbody>
<tr>
<td>72 (37-89)</td>
<td>7/40</td>
<td>263 (104-1074)</td>
<td>2.18 (0.69-6.19)</td>
<td>0.19 (0.06-1.50)</td>
<td>38</td>
<td>20</td>
<td>60</td>
<td>100</td>
<td>100</td>
<td>0%</td>
<td>40%</td>
<td>60%</td>
</tr>
</tbody>
</table>

Results: Main clinical characteristics are summarized in table 1. 85% were females, median age was 72 years (37-89) and most of patients (95%) had del(5q) as the only cytogenetic abnormality. Among 47 patients, only 38 were evaluable at week 12 (6 out of 38 discontinued the study: 3 due to DP, 1 due to toxicity and 1 for unknown reasons). 7 patients are currently receiving the first 12 weeks of treatment and 2 patients were excluded (screening failures). Regarding efficacy (w12), data from 36 patients were available. Hi-ER was observed in 14/36 patients (39%), minor Hi-ER (Hb increased<1.5g/dL) in 4/36 (11%), stable disease in 15/36 (42%) and PD (transfusion dependency) in 3 (8%). CyR was available in 30 patients: complete CyR was obtained in 12 (40%), partial CyR in 6 (20%) and no CyR in 12 (40%) patients. Safety information in 38 patients demonstrated that most patients (87%) developed any adverse events (AE) while only 42% of these were relevant (G3-4). Most G3-4 AE were hematological (neutropenia 38%) being non-hematological only in 6%. Seven serious AE were reported in 5 patients: vestibular neuritis, congestive heart failure, polyarthritis, arterial hypertensive crisis, carpal arthritis, respiratory infection and chronic obstructive pulmonary disease exacerbation. All SAE were not related with the drug of the study (LEN/Placebo).

Summary/Conclusions: In this study we confirm a high rate of erythroid and cytogenetic responses early after treatment with an adequate safety profile in the first 12 weeks of treatment with LEN or placebo.

E1182

MYELODYSPLASIA-RELATED MORTALITY REMAINS THE MAIN CAUSE OF DEATH ALONG DIFFERENT GROUPS OF RISKS: AN ANALYSIS FROM MDS ARGENTINEAN STUDY GROUP

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Background: Myelodysplastic syndrome (MDS) are the most frequent hematological malignancy in elderly patients. The impact of MDS burden over overall mortality remains controversial, moreover, after the incorporation of hypometabolizing agents in the therapeutic armamentarium.

Aims: We aimed to analyze overall mortality and causes of death in our population of patients with MDS.

Methods: A retrospective analysis of patients with MDS reported to Argentinean MDS registry and a previous study from Academia Nacional de Medicina. Causes of death were classified in: acute myeloid leukemia (AML), infections, bleeding, solid tumor, cardiovascular, transplant related mortality (MRT), others and unknown. AML, infections and bleeding were considered as MDS-related mortality. Causes of death were analyzed using cumulative competitive events curves with Gray test and Fine-Gray for proportional hazard regression was used for the multivariate analysis.

Results: From 1981 to 2016, 1040 patients with MDS were recorded; 717 out of 1040 (69%) were diagnosed after 2006. Median age of patients was 70 years (range: 14-96 years) with 588 (56%) being males. MDS was primary in 574 patients (94%). Median follow-up of 25 months (range: 1-170 months) for the surviving patients. The cumulative incidence of overall mortality was 20% at 12 months (95%CI 2-22%), 37% at 24 months (95%CI 3-40) and 59% at 60 months (95%CI 5-63). The incidence of overall mortality did not significantly differ along the different groups of diagnosis (p=0.291) neither according to age group. Multivariate analysis for cumulative incidence of overall mortality found Charlson index (HR 1.38; p<0.001), sex (HR 1.45; p=0.014) and IPSS-R (HR 2.79; p=0.001) as prognostic variables. The main cause of death was AML accounting for 9% at 12 months (95%CI 7-11), 16% at 24 months (14-19) and 25% at 60 months (95%CI 22-28) of mortality for all patients. Infection-mortality and bleeding-mortality were the second and the third cause of death respectively. MDS-related mortality was 16% at 12 months (95%CI 13-18), 29% at 24 months (95%CI 26-32) and 44% at 60 months (95%CI 40-48); this incidence was not different by year of diagnosis. MDS-related mortality remained the main cause of death in all IPSS-R groups and in all Charlson index categories. Multivariate analysis for cumulative incidence of MDS-mortality found Charlson index (HR 1.29; p=0.02), IPSS-R (HR 2.88; p<0.001) and sex (HR 1.47;p=0.03) as independent variable. Age (p=0.034) and IPSS-R (p<0.001) were associated with AML-related mortality. A total of 56 patients underwent allogeneic transplant; cumulative incidence of MRT for all cohort was 0.5% at 12 months (95%CI 0.2-1.2) and 1.4% at 24 months (95%CI 0.8-2.4). Only male sex was associated with a higher cumulative incidence of mortality by solid tumor (p=0.001) and a Charlson index ≥2 was associated with higher cumulative incidence of cardiovascular mortality (p=0.021).

Summary/Conclusions: In this large cohort of patient with MDS we demonstrate that MDS-related causes are the leading cause of death along all IPSS-R groups. The absence of difference in mortality along the years of diagnosis highlights the necessity of better treatments for these patients.
was evaluated by at least two morphology experts and a consensus diagnostic of MDS-confirmed, MDS-suspected or MDS-excluded was emitted. MFC was performed applying at least five-color staining and a numerical score was calculated for every patient following criteria defined by Ogata et al (Blood. 2006 Aug 1;108(3):1037-44), with a score ≥2 suggesting MDS. Conventional karyotype and FISH employing probes to detect usual 5q-, 7q-, +8, 20q- and del(17p) in cytogenetic abnormal cases.

Results: Median age of the patients was 73.7 y/o. Patients presented with anaemia in 88% (84%), neutropenia in 36 (34%) and thrombopenia in 49 (47%). Cytomorphology was reported as MDS-confirmed (60 pts), MDS-excluded (22) or MDS-suspected (23). MDS subtypes were Multilineage Dysplasia (23), Unityne Dysplasia with Ring Sideroblasts (9), del(5q) Syndrome (3) and Unclassified (2). 4 pts being diagnosed of CMMML. MFC score was MDS-suggestive in 56 cases, MDS-not suggestive (36) and in 13 cases its use was precluded because of morphology findings. Considering cytomorphology as gold standard, MDS-related were patients with MDS confirmed by morphology. MFC score sensitivity was 77%, specificity 88%, with positive and negative predictive values of 96% and 56% respectively. Furthermore, MFC score showed a significant correlation with single morphologic findings of granulocytic (p<0.001), erythroid (p=0.001) and megakaryocytic dysplasia (p=0.002), and a trend toward significant association with del(7q) by FISH (p=0.085). In the subset of patients with MDS-suspected but not confirmed by morphology, the presence of a MFC score ≥2 was significantly associated with a poorer overall survival (log-rank p=0.012), with all MFC score ≥2 patients alive after a median follow-up of 35 months. There was also a trend to statistical association between MFC score and overall survival in the whole series of patients (log rank p=0.053). Interestingly, there was a striking difference in risk of evolution to AML according to MFC findings (log rank=0.013), with a 100% of patients free from this complication in the group of patients with MFC score ≥2.

Summary/Conclusions: MFC analysis of the bone marrow provides useful information in the diagnosis of MDS which can be specially helpful in the subset of patients with inconclusive morphological findings, showing a strong correlation in this group of patients with clinical outcome in terms of risk of evolution to AML and overall survival.

E1184 ECONOMIC IMPACT AND HEALTHCARE UTILIZATION IN PATIENTS WITH HIGHER-RISK MYELODYSPLASTIC SYNDROMES (HR-MDS) IN ROUTINE CLINICAL CARE IN THE UNITED STATES (US) – A CLAIMS DATABASE STUDY

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Background: Therapy for patients with HR-MDS includes systemic chemotherapy, stem cell transplant (SCT), and supportive care aimed at improving symptoms associated with MDS-related disruption of normal hematopoiesis. However, the economic impact of these interventions over time for HR-MDS patients has not been fully examined.

Aims: We evaluated the costs and healthcare utilization (HCU) of US HR-MDS patients treated during routine care.

Methods: Newly diagnosed adult HR-MDS patients who initiated first-line therapy were identified from Optum, a large US claims database, between 1/1/08 and 10/31/15. HR status was based on ICD coding: ≥1 inpatient claim or ≥2 outpatient claims with ≥1 HR-MDS (ICD-9 code: 239.73; ICD-10 codes: D46.20, D46.21, D46.22). The first MDS claim served as the index date. Exclusion criteria included: lack of continuous enrollment in medical/pharmacy benefits in the 12 months prior to index date (baseline period), presence of other primary cancer, or receipt of any chemotherapy or SCT during baseline period. MDS-related and non-MDS-related HCU and costs incurred during follow-up were evaluated. MDS-related HCU and costs were medical claims with usual 5q-, 7q-, +8, 20q- and del(17p) in cytogenetic abnormal cases.

Results: 209 treated HR-MDS patients were identified. During the follow-up period, 69.4% of patients had ≥1 inpatient admission, but more patients had an MDS-related than non-MDS-related admission (Table 1). 56.9% of patients had MDS-related, 1 and non-MDS-related HCU and costs incurred during follow-up were evaluated. MDS-related HCU and costs were medical claims with usual 5q-, 7q-, +8, 20q- and del(17p) in cytogenetic abnormal cases.

Summary/Conclusions: The economic impact of HR-MDS is considerable, with higher costs incurred within the first year of diagnosis. The decrease in cost between Year 1 and Year 2 was mainly due to decreased MDS-related medical costs. Consistent with this cost trend, healthcare utilization for MDS-related services decreased in Year 1 vs Year 2. As treatment of HR-MDS continues to evolve, economic impact and HCU need to be further investigated in this patient population.

E1185 INTRAVENOUS IMMUNOGLOBULIN IS AN EFFECTIVE TREATMENT FOR CYTOPENIAS ASSOCIATED TO CIRCULATING T-CELL CLONES IN MYELODYSPLASTIC SYNDROMES

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Background: Myelodysplastic syndrome (MDS) can be associated with immunologic disorders, including autoimmune cytopenias and Coombs positive or negative (C+) hemolytic anemia. Abnormally expanded T-cells can be detected in these patients, possibly contributing to both bone marrow insufficiency and immunologic disorders, and a target for therapeutic intervention.

Aims: To explore the role of intravenous immunoglobulin (IVIG) as a treatment for immune-related cytopenias in a series of 20 consecutive patients with MDS at a single institution.

Methods: T-cell clonal expansion in the peripheral blood (PB) was documented by flow cytometry and PCR. Eighteen patients had a confirmed MDS (16 IPSS lower-risk, LR). Two suspected MDS were designated as idiopathic cytopenia of uncertain significance (ICUS). Reasons for IVIG treatment were chronic hemolysis refractory to corticosteroids (16: 12 LR, 1 higher-risk (HR), 1 ICUS) or pancytopenia (2 LR and 1 HR refractory to standard therapy, 1 ICUS) associated to T-cell clonal proliferation in the PB. Hematological response was assessed by IWG criteria 2006. Hemolysis response (HLR) included normalization (CR) or a greater than 50% improvement (PR) of LDH, reticulocytes, indirect bilirubin and haptoglobin.

Results: Clinical characteristics are shown in the Table. All patients had a chronic T-cell proliferation (PCR) over the follow-up period in 12/13 patients (92%). In 9 cases the clone was characterized by flow cytometry: 6 had a CD3+ T-cell and 3 had a CD3+CD16+CD56+ NK-cell expansion. Associated immunologic disorders were: ITP (4), neutrophil dermatosis (3), inflammatory bowel disease
Summary/Conclusions: Treatment with IVIG of C3 hemolytic anemia and pancytopenia associated with T-cell immune-clones and MDS was safe and yielded high rates of durable response on all linesages and on hemolysis. Transfusion independency and reduction/discontinuation of corticosteroids for chronic hemolysis make this drug a valuable option not only in LR but also in HR patients, although a confirmation on larger cohorts is needed. IVIG at intermediate-high dose suppresses proliferation of T-cells and induces immune-regulation. Given the relative rarity of T-cell clones in MDS, further investigational studies are underway to define their pathogenetic role and the mechanism of action of IVIG in this specific subset of patients.

E1186 DEVELOPMENT AND EXTERNAL VALIDATION OF A NEW PATIENT-CENTERED PROGNOSTIC INDEX FOR PATIENTS WITH ADVANCED MYELODYSPLASTIC SYNDROMES

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Background: The clinical presentation of myelodysplastic syndromes (MDS) is highly variable, and the ability to accurately predict outcomes is critical. Current prognostic systems for these diseases are based on traditional clinical, pathologic and laboratory indicators.

Aims: We aimed to develop and validate a new prognostic index for advanced MDS by including self-reported fatigue severity into a well-established clinical risk classification: the International Prognostic Scoring System (IPSS).

Methods: Untreated patients (n=280) were recruited at the time of diagnosis of advanced MDS from 37 hospitals in nine countries to create the index. The index was then applied to an independent cohort including pre-treated MDS patients from the Dana-Farber Cancer Institute (DFCI) in Boston, Massachusetts (USA; n=189). Patients in both the International and DFCI cohorts were adults with newly-diagnosed intermediate-2 or high-risk MDS (advanced disease on the IPSS). Patients were enrolled regardless of age, comorbidity, performance status and prognosis, to create a lower IPSS risk category. All completed a baseline health-related quality of life assessment. Data from international and DFCI cohorts were independently collected and analyzed. Univariate and multivariate Cox proportional hazards (PH) regression analyses were performed to estimate hazard ratios with 95% Confidence Intervals (CIs). Discrimination and calibration were evaluated for both the development (internal validation) and independent DFCI datasets (external validation). Statistical significance for all tests was set as two-sided p<0.05.

Results: A new risk classification was developed, namely, the fatigue (FA)-IPSS(h). Whereas use of the standard IPSS in more advanced disease discriminates between two risk categories for untreated patients, the new fatigue FA-IPSS(h) classification was able to distinguish three survival groups with distinct survival outcomes. Overall survival rates at 6 months, 1 year, and 2 years were markedly different among the three groups. To illustrate, one year survival was 80.3% (95% CI, 73.4-87.8), 60.5% (95% CI, 52.3-70.0) and 37.6% (95% CI, 23.9-59.1) for patients classified into Risk-1, Risk-2 and Risk-3 respectively. Median OS in DFCI data by FA-IPSS(h) risk was similar to that of the development cohort for each of the three risk groups, indicating good external calibration. Patterns of OS through 2 years were also distinctly between risk groups as in the development cohort of untreated patients, with one exception: 2-year OS was similar for FA-IPSS(h) risk-3 and risk-2. Predictive accuracy of this new index was higher than the IPSS alone in both the development cohort (C-statistic, 0.65 vs 0.57) as well as in the independent cohort including pre-treated patients (C-statistic, 0.58 vs 0.54).

Summary/Conclusions: The FA-IPSS(h) is an additional prognostic tool that might enhance clinicians’ ability to provide more personalized treatment strategies both in untreated and pretreated advanced MDS patients. This analysis offers a model for integration of PROs in prognostic systems for patients with other cancers and advanced illnesses.
Results: Overall, no significant correlation was seen between expression of p53 and degree of fibrosis (p=0.25). However, degree of fibrosis predicted for overall survival in patients with p53 expression (median overall survival of 4 months in patients with both p53 over expression and significant fibrosis compared with median overall survival of 18 months in patients with p53 over expression without fibrosis, p<0.001). In patients who received azacitadine, though there was significant fibrosis and p53 over expression had a significantly increased overall survival compared with those who did not receive azacitadine (4 month versus 1 month, p=0.002). Azacitadine treatment was not associated with increased survival in patients with p53 expression without fibrosis but these patients did have an overall increased survival compared to those with fibrosis (median survival 12 vs 37 months).

Summary/Conclusions: This study confirms that significant marrow fibrosis adversely affects overall survival in patients with MDS, including those with p53 over expression. Patients who received azacitadine had a significant increase in median survival. Although the numbers of patients who received azacitadine with significant fibrosis does not suggests that patients with fibrosis may benefit from the use of azacitadine and larger and randomized studies should be considered to study this further.

References

E1189
SUCCESSFUL TREATMENT WITH DANAZOL FOR MYELODYSPLASTIC SYNDROMES AND APLASTIC ANEMIA REFRACTORY OR INELIGIBLE TO STANDARD THERAPY
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Background: The discovery of danazol potential activity on telomere elongation in bone marrow failure has renewed interest in this drug. The treatment of cytopenia in myelodysplastic syndromes (MDS) and aplastic anemia (AA) patients who fail or are ineligible to standard therapies is an unmet medical need; however only dated reports on danazol use in this setting are available. Aims: We report the results of treatment with danazol in patients with MDS and AA at a single institution (2015-2020).

Methods: From June-11 to May-15, danazol was administered to 31 consecutive patients (20 MDS and 11 AA). Criteria for treatment were non-severe AA (8), severe AA ineligible/refractory to immunosuppressive therapy or allogeneic transplantation (3), transfusion dependent (TD) lower risk MDS refractory to azacitadine and lenalidomide (11), MDS with isolated thrombocytopenia <50x10^9/L (6) or with bone marrow hypoplasia and bicytopenia (3). Diagnosis was defined by WHO 2008 for MDS and according to Camitta (Blood 1975) for AA; response was evaluated by IWG 2006 criteria.

Results: The characteristics of the patients are shown in the Table. All MDS patients except 1 had low-risk disease according to IPSS and IPSS-R. The response was complete or partial, except 2 and 3 patients respectively. Nineteen patients (12 MDS, 7 AA) received danazol at full dose (600mg daily). A 400mg daily dose was given to 12 patients, due to toxicity (4 MDS, 4 AA) or comorbidities (4 MDS). Median duration of treatment was 22 months (range 1-44 months).

Summary/Conclusions: We found that one quarter of all patients who underwent a FISH panel workup for a suspected diagnosis of MDS presented with aberrations in at least one of the four selected probes, a proportion which was significantly lower (one fifth) when a full sample was analyzed, and significantly higher (one third) in FACS purified blast cells. Although the purification of the sample through FACS doubled the percentage of positive cells within each sample for all four probes, the likelihood of obtaining a positive result for del(7q) and T8 in the cohort was unaffected by the methodology used. In contrast, the use of a sorted sample greatly increased the proportion of positive findings in del(5q) and, especially, in del(20q), the two probes for which the basal positivity in full samples were lowest. The clinical value of this increased rate of detection of del(5q) and del(20q) remains unclear, since their prognostic utility has only been established at levels detectable by conventional karyotyping of a full sample.

E1188
FACS PURIFICATION OF BLAST CELLS IN MDS IMPROVES THE DETECTION RATE FOR DEL(5Q) AND DEL(20Q), BUT NOT FOR DEL(7Q) OR T8
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Background: Prognostication in Myelodysplastic Syndromes (MDS) using validated scores includes the detection of chromosomal aberrations by conventional karyotyping. When the latter is unavailable or unsuccessful, fluorescence in-situ hybridization (FISH) panels can be used. Although panels vary by laboratory, some of the most commonly used probes include the search for monosomy 5 or del(5q), monosomy 7 or del(7q), del(20q) and trisomy 8 (T8). In our laboratory, FISH was historically performed on full samples, i.e. unsorted or after FACS (95%). Since 2015; we have primarily performed the analysis on Fluorescence Activated Cell Sorting (FACS) separated blast cells.

Aims: In this study, we aim to analyze the benefit of using purified samples of blast cells for FISH analysis in MDS, when compared to full mixed cellularity samples.

Methods: We reviewed all samples analyzed in our laboratory between January 1st 2011 and February 28th 2017 in which a FISH panel was performed due to a suspicion of myelodysplasia, using probes for del(5q), del(7q), del(20q) and T8. The proportion of patients positive for the test, as well as the proportion of positive cells within a positive sample, were compared.

Results: We obtained valid results for 328 samples during the relevant time-frame, 39.6% of which were collected from female patients. FISH was performed after FACS in one third of samples (35.1%, n=115), starting in 2015. Considering the overall cohort, nearly a quarter of samples (23.8%) had at least one aberration in the four probes tested in this study. This proportion of aberrations was significantly higher in double in FACS compared (33.0%) versus full sample patients (18.8%, p=0.004). Del(5q) was present in 5.6% of the cohort; however, positivity was 5-fold higher in FACS patients, compared to full sample patients (12.3% vs 1.6%, p<0.001). Considering the percentage of positive cells in each sample, it doubled from 38.7±29.9% in the full sample to 71.6±38.1% after FACS, p<0.001. Del(7q) was similarly present in 5.7% of the cohort; however, in contrast, there were no relevant differences between FACS patients, 4.2% of whom had del(7q), and full sample patients (8.1%, p=NS). There were, however, differences in the percentage of positive cells within the sample, doubling from 32.1±11.2% in the full sample to 77.6±17.8% after FACS, p<0.001. Del(20q) was similarly present in 4.0% of the cohort; however, positivity was significantly lower (one fifth) when a full sample was analyzed, and significantly higher (one third) in FACS purified blast cells. Although the purification of the sample through FACS doubled the percentage of positive cells within each sample for all four probes, the likelihood of obtaining a positive result for del(7q) and T8 in the cohort was unaffected by the methodology used. In contrast, the use of a sorted sample greatly increased the proportion of positive findings in del(5q) and, especially, in del(20q), the two probes for which the basal positivity in full samples were lowest. The clinical value of this increased rate of detection of del(5q) and del(20q) remains unclear, since their prognostic utility has only been established at levels detectable by conventional karyotyping of a full sample.

Summary/Conclusions: This study confirms that significant marrow fibrosis adversely affects overall survival in patients with MDS, including those with p53 over expression. Patients who received azacitadine had a significant increase in median survival. Although the numbers of patients who received azacitadine with significant fibrosis does not suggests that patients with fibrosis may benefit from the use of azacitadine and larger and randomized studies should be considered to study this further.

References
was 19 months (mo) (1-66) in AA and 6 mo (1-60) in MDS. ORR was 73% and 50%, respectively. Age and hemoglobin levels impacted on response in AA. Hematological improvement was seen on all lineages in 92% of cases, with a median time to best response of 3-5 mo on platelets and neutrophils and of 8-12 mo on hemoglobin. Interestingly, duration of response in MDS patients was significantly longer with a danazol dose of 600mg than with 400mg (p<0.001). Conversely, dosing did not impact on response to danazol in AA patients. Grade 2-3 toxicity was significantly higher in AA patients (p<0.05), 60% pretreated with IST. Adverse events included: hepatotoxicity (3 G1, 1 G2, 3 G3), muscle pain/CPK elevation (3 G1, 2 G2), transient renal impairment (1 G1), hypoxemia (1 G1). Responders to danazol had a better survival in terms of OS and EFS in both groups (Figure 1).

Table 1.

<table>
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<tr>
<th>AA</th>
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<td>Cytopenia (%)</td>
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**Figure 1.**

**Summary/Conclusions:** Danazol was proved both effective and safe as treatment of cytopenia in MDS and AA patients refractory or ineligible to standard therapies. The daily dose of 600mg was more effective for MDS patients, whereas a lower dose of 400mg may have a better risk/benefit ratio in AA. Younger AA patients with less severe anemia were more likely to respond. Danazol use is particularly attractive in thrombocytopenic patients, where responses were rapid, but delayed responses may be expected also on anemia by using dana-
zol for prolonged periods, when tolerated. Response to danazol is also poten-
tially associated to a survival advantage, although these data should be con-
firmed by larger prospective studies.

E1192

**DOSE-CONFIRMATION PK/PD STUDY OF ORAL ASTX727, A COMBINATION OF ORAL DECITABINE WITH A CYTIDINE DEAMINASE INHIBITOR (CDAI) E7727, IN SUBJECTS WITH MYELODYSPLASTIC SYNDROMES (MDS)**


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**Background:** We have previously shown that ASTX727, a combination of oral decitabine and the oral CDAI E7727, emulates the pharmacokinetics of a one hour intravenous decitabine infusion (IV-DAC) in a dose-escalation phase 1 study. (Garcia-Manero. Blood 2016 128: 114)

**Aims:** To confirm pharmacokinetic (PK) and pharmacodynamic (PD) compa-
rability of 20mg/m² IV-DAC administered D1-5 of a 28 day cycle with an entire cycle of ASTX727 given at the selected dose from phase 1 (35mg decitabine and 100mg of E7727).

**Methods:** Adult patients with Int-1/int-2 or HR MDS or Chronic Myelomonocytic Leukemia (CMML) were enrolled in a randomized cross-over Phase 2 study. Patients were randomized 1:1 to receive in the first 28 day cycle, either 5 days of IV-DAC 5 days of ASTX727, followed by a cross-over to the other in Cycle
2. Cycles 3 forward were with ASTX727. PD and clinical response were assessed on all patients. Summary/Conclusions: Patients treated with azacitidine (especially APL patients) did not experience infection at high risk of infection during the first AZA course. All important predictive factors should be assessed before therapy. Patients possessing factors predictive for infection require special approach and predictive infection model should be developed in further analysis.

E1194
OVERALL SURVIVAL, INITIAL TREATMENT AND TREATMENT DURATION OF PATIENTS WITH MYELODYSPLASTIC SYNDROME, A DETAILED POPULATION BASED STUDY
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Background: Population-based studies on myelodysplastic syndrome (MDS) containing detailed clinical information of patient characteristics, treatment and follow-up of the disease are scarce. Since 2005, all patients diagnosed with hematological malignancies in Friesland, a province in the Netherlands, are prospectively registered and followed by their clinicians in a population-based registry, the HemoBase. The registry provides representative population-based data on diagnosis, treatment and outcomes in an era where low-intensity treatment such as hypomethylating agents have become available for the elderly.

Aims: The objectives of this study were to determine the overall survival (OS) of patients with MDS and the effect of the variables gender, age, comorbidities, IPSS, IPSS-R and MDS subtype according to WHO 2016 classification. Furthermore, the leukemia free survival (LFS), the initial treatment and the duration of first-line treatment were analyzed.

Methods: An observational, population-based study was performed using the HemoBase registry. The bone marrow biopsies and aspirates of all MDS patients treated between 01-01-2005 and 31-12-2013 were independently and blindly reviewed by both the hematologist and hematologist-pathologist and classified according to the WHO 2016. Treatment categories were defined as intensive chemotherapy (IC) either combined or not combined with allogeneic stem cell transplantation, the hypomethylating agent azacytidine, the immunomodulatory agent lenalidomide, hydroxyurea or best supportive care (BSC) (blood transfusions, erythropoiesis-stimulating agents). Approval was obtained from the Medical Ethics Review Committee from Medical Centre Leeuwarden. Statistical analyses were performed with SPSS 19; survival analyses were used presenting Kaplan-Meier estimates.

Results: 217 patients (72.4% male, 66.8% >70 years old, median age 75 years, 27.2% Charlson Comorbidity Index (CCI) score ≥3) were included with a median follow-up duration of 70.2 months. 15.7% of the population had an IPSS score of ≥1.5 and 12.4% of the population had an IPSS-R score of ≥4.5. In 41.5% no cytogenetic information was available. MDS-RS, MDS-SLD-MLD, MDS-EB, MDS-U and CMMML were diagnosed in 11.5%, 14.7%, 36.4%, 27.2% and 10.1% of the population respectively. 18.4% showed progression towards acute myeloid leukemia (AML), IC, azacytidine, lenalidomide, hydroxyurea and BSC were the initial treatment in 5.1%, 13.8%, 1.4%, 9.7% and 66.4% of the patients respectively. Within 12 months 78.1% of all treated patients terminated their first-line therapy because of death (20.0%), refractory to treatment (18.3%) or disease progression (16.7%). A second treatment was initiated in 10.1% of patients. The median LFS was 18.2 months (95% CI: 12.6-23.8). The median OS of MDS patients in Friesland was 22.5 months (95% CI: 15.2-29.7). Univariate analysis showed an association between lower OS and male gender (HR for women: 0.54, p=0.008, 95% CI: 0.34-0.85), age >80 years (HR: 2.7, p<0.005, 95% CI: 1.6-4.6, CCI score ≥3 (HR: 2.0, p<0.001, 95% CI: 1.3-3.0). IPSS score ≥1.5 (HR: 2.3, p=0.004, 95% CI: 1.3-4.1). IPSS-R score ≥4.5 (HR: 5.7; p<0.0005, 95% CI: 2.4-2.4) and MDS subtype MDS-EB (HR: 1.8; p=0.016, 95% CI: 1.1-2.9).

Summary/Conclusions: This study provided complete representative population-based data on overall survival and treatment of patients with MDS at a median follow-up duration of 70.2 months. Despite the limited comorbidity in this population, a third of the patients received treatment in addition to BSC.

E1195
DANAZOL TREATMENT FOR THROMBOCYTOPENIA IN LOWER-RISK MYELODYSPLASTIC SYNDROMES: A REAL LIFE EXPERIENCE
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Background: Severe thrombocytopenia is an uncommon event in lower-risk MDS patients, but it may significantly affect the prognosis. In fact, when it occurs, the treatment of thrombocytopenia with antithrombotic agents may not be sufficient. Danazol, a synthetic androgen, is a drug already used in the treatment of androgen deficiency and infertility but its potential role in reducing bleeding is still under debate. Several studies suggested it is effective in this setting, but its real world effectiveness in reducing bleeding is still under debate. Our study aimed at evaluating the real-life effectiveness of danazol in reducing bleeding in a cohort of low-risk MDS patients.

Methods: A retrospective cohort study was performed on 24 consecutive patients treated with danazol for platelet levels ≥50G/L and platelet count decrease of ≥50% at any point in time. The cohort was evaluated for a period of 12 months from the start of the treatment, defined as the period of time after the platelets count decrease of ≥50% was observed. The main outcomes were the platelet count at the end of the observation period and the number of bleeding events during this period.

Results: At the end of the observation period, the median platelet count was 130G/L (IQR 90-190G/L). The median number of bleeding events was 3 (IQR 1-6). The median time to achieve platelet count recovery was 4 months (IQR 2-6). No adverse events were reported.

Conclusion: Danazol is an effective treatment for thrombocytopenia in low-risk MDS patients. It is a safe and well-tolerated drug with a beneficial effect on platelet count and bleeding rate. Despite the limitations of our study, this finding suggests that danazol could be a useful addition to the treatment of thrombocytopenia in low-risk MDS patients.
suitable, but, at present, its safety is questioned in MDS patients. Furthermore, in clinical practice, danazol, an attenuated androgen, has been reported to have some ability to increase the platelet count in this context (Wattel 1994; Chan 2002).

Aims: To assess efficacy and toxicity of danazol employed to improve severe thrombocytopenia in lower-risk MDS setting.

Methods: We retrospectively reviewed twenty-four patients affected by MDS and treated with danazol for thrombocytopenia. The initial dose was 600mg/day for all patients. The IWG criteria of response (Cheson 2006) were adopted. The outcome was observed every 3 months till 12th month. The overall response rate and the average platelet count or each time of observation were described. Progression free survival was estimated with the Kaplan-Meier product limit method, followed by the logrank test and by the Cox proportional-hazard regression.

Results: Of the 24 patients, 3 patients had a therapy-related MDS. At the starting time of danazol therapy, the IPSS was “low” in 9, “int-1” in 13 and “int-2” in 2 cases respectively; the IPSS-R was “very low” in 2, “low” in 11, “intermediate” in 7 and “high” or “very high” in 4 cases. At baseline in 14 patients the platelet count was lower than 20x10^3/mL, the average was 20x10^3/mL and the maximum value was 38x10^3/mL. The median dose was 600mg (range 200-600) also maintained at least up to 3 months (range 400-600). At 6 and 12 months the median dose therapy was 400mg (range 400-600 and 200-600 respectively). The response rate was 79.1% (19 responders on 24 treated). The average count increased as shown in Figure 1, over 60x10^3/mL after 6 months from the beginning of therapy and so maintained after one year. Only 3 patients lost the response at 187, 600 and 633 days respectively. The median survival was not reached in the presented series, and the probability to maintain the response was over 75% after two years from the beginning therapy in the responder patients (Figure 2). Adverse events recorded were as follows: moderate (grade 3) (with subsequently drug suspension); severe (grade 3) but reversible renal failure in 1 case (the drug was stopped); moderate (grade 1 and 2) increase in transaminases in 4 cases (with reduction of danazol to 400mg/day in 2 of these); 1 case of severe but reversible liver toxicity (grade 1 and 2) (with reduction of danazol to 400mg/day in 2 of these); reversible cutaneous rash in 3 cases; amenorrhea in 1 case (the only fertile woman in the series); weight loss and loss of appetite in 1 case, weight gain in 1 case.

Summary/Conclusions: This series confirms the efficacy of danazol to improve platelet count in the most of patients with severe thrombocytopenia due to lower-risk MDS. In all patients with increased platelet count, the response was clinically significant. The median dose of 600mg should be maintained for at least 3 months to properly assess the effectiveness of therapy and then adjusted according to response and toxicity. The response may not be immediate, but seem to be reachable after 3-6 months of treatment. A responsive patients have short probability to lose the response, that may last for very long time. The toxicity profile of this drug is low. The mechanism of action of danazol in MDS patients remains unclear. Waiting for more information on the efficacy and safety of eltrombopag from the clinical trials in progress, danazol may be a good therapeutic option for these patients.

E1196

TREATMENT PATTERNS IN PATIENTS WITH HIGHER-RISK MYELODYSPLASTIC SYNDROMES (HR-MDS) IN ROUTINE CLINICAL CARE IN THE UNITED STATES (US) – A CLAIMS DATABASE STUDY

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Background: Treatment of patients with HR-MDS includes hypomethylating agents (HMAs) (azacitidine and decitabine), high-intensity induction chemotherapy (IC), and stem cell transplant (SCT). Given the rarity of disease, information available on how these treatments are applied in practice is limited.

Aims: We evaluated the treatment patterns of HR-MDS patients engaged in routine care within the US.

Methods: Newly diagnosed HR-MDS patients who were ≥18 years old were retrospectively identified from Optum, a large US claims database between 1/1/2008 and 10/31/2015. HR status was based on ICD coding: ≥2 inpatient claim or ≥2 outpatient claims with an HR-MDS ICD-9/10 code (ICD-9 code: 238.73; ICD-10 codes: D46.20, D46.21, D46.22). The first MDS claim served as the index date. Exclusion criteria included: absence of continuous enrollment in medical and pharmacy benefits for 12 months prior to index date (baseline period), presence of other primary cancer, or receipt of any chemotherapy or SCT during the baseline period. First-line therapy (1LT) was defined as an MDS-specific treatment (as defined by NCCN MDS Guidelines v2.2017) initiated on or after the index date. Patients were followed until death, end of continuous enrollment, or end of study (12/31/2015). For patients with progression to acute myeloid leukemia (AML), treatment pattern evaluation stopped at AML diagnosis.

Results: 335 newly diagnosed HR-MDS patients were identified; 209 (62.4%) were treated with 1LT with treatment initiated within 1 month of diagnosis (median: 17 days, interquartile range [IQR]: 9, 35). A higher proportion of untreated patients (n=126) was ≥75 years of age (71.4% vs 53.1%) and had certain comorbidities at baseline (congestive heart failure, 23.0% vs 16.3%; renal disease, 24.6% vs 16.3%; diabetes 31.0% vs 23.4%, diabetes with end organ failure, 16.7% vs 8.1%) than treated patients. For treated patients, 1LT with azacitidine predominated in 68.9% of patients (n=144), followed by decitabine in 20.6% of patients (n=63), and immunomodulators (lenalidomide or thalidomide) in 8.7% of patients (n=18) (Figure 1). 4 patients had only SCT and an additional 14 had SCT at some point during follow-up. With regard to HMA therapy, median duration was 4.5 months (IQR: 2.6, 9.5) for azacitidine and 4.8 months (IQR: 2.1, 11.6) for decitabine. A greater proportion of decitabine-treated patients
received supportive care with colony-stimulating factors (CSFs) (39.5% vs 28.5%) and either erythropoietin or platelet transfusions (69.8% vs 57.6%) during 1LT vs azacitidine-treated patients. Second-line therapy (2LT) was administered to 30 (14.4%) patients; the HMAs again predominated in 63.3% of patients (n=19). Of patients not receiving 2LT, 65 (31.7%) progressed to AML, 47 (22.9%) had <30 days of follow-up due to proximity to end of study (38 [80.9%] of these were on 1LT at last evaluation), 33 (16.1%) continued to receive some supportive care and, 21 (10.2%) died.

Summary/Conclusions: Most HR-MDS patients treated in routine care are treated according to guidelines, with the HMA, azacitidine, predominating. Underlying comorbidities and older age may influence whether or not to treat HR-MDS patients with 1LT. For treated HR-MDS patients, duration of 1LT did not differ with azacitidine and decitabine. However, use of certain MDS-related supportive care treatments varied by choice of HMA, with more decitabine-treated patients receiving CSFs and transfusions. Further research is needed to determine how these factors influence both clinical outcomes in a HR-MDS population.

E1197
APPREC8: A PIPELINE FOR PRECISE VARIANT CALLING INTEGRATING 8 TOOLS
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Background: For the use of next-generation sequencing in clinical routine valid variant calling results are crucial. However, evaluation of eight open-source variant calling tools on different datasets has pointed out that variant calling - even of single nucleotide variants and short indels - remains challenging. Perfect results could not be obtained with any of the considered tools. High sensitivity was always accompanied by low positive predictive value (PPV).

Aims: We aimed at developing a variant calling pipeline with both, high sensitivity and high PPV.

Methods: We developed appre8, a variant calling pipeline combining the output of eight open-source variant calling tools: GATK HaplotypeCaller, Platypus, VarScan, LoFreq, FreeBayes, SNVer, SNaTtools and VarDict. The pipeline performs several steps of filtration, including a final automatic characterization of all reported calls as artifacts, likely polymorphisms and likely mutations. To train our pipeline, we analyzed two data sets covering data of 54 myelodysplastic syndrome (MDS) patients, sequenced on Illumina HiSeq, and 111 MDS patients, sequenced on Roche 454. In all cases the same target region consisting of 19 genes (42,322bp) was analyzed. Validation was performed by re-sequencing on the same platform, on a different platform and expert-based review.

Results: When analyzing the training sets with only one of the eight variant calling tools and considering all variants - pathogenic as well as somatic - sensitivity ranges between 0.85 and 1.00 in case of set 1 and 0.47 and 0.99 in case of set 2. Although FreeBayes features highest sensitivity regarding both sets, it consistently features lowest PPV as well (set 1: 0.03, set 2: 0.02). Combining the output of all variant calling tools leads to perfect sensitivity, while PPV is 0.03 for set 1 and 0.02 for set 2. Application of our appre8 pipeline leads to a minor decrease in sensitivity (set 1 and set 2: 0.98), while PPV is significantly increased (set 1: 0.99, set 2: 0.94). The PPV of the appre8 output for both training sets is higher compared to each of the individual tools. Analysis of the independent test set 1 leads to comparable results. Sensitivity of the individual tools ranges between 0.82 and 0.99, while PPV ranges between 0.02 and 0.91. Combining the output of all variant calling tools leads to sensitivity of 1.00 and PPV of 0.02. However, application of appre8 leads to variant calling results with sensitivity of 0.98 and PPV of 0.99. To test the robustness of our approach, we analyzed Roche 454 data, although the pipeline was exclusively trained on Illumina data. Regarding the individual tools sensitivity ranges between 0.91 and 0.99, while PPV ranges between 0.07 and 0.68. By combining the output of all variant calling tools, sensitivity increases to 0.99, while PPV is 0.05. Application of appre8 leads to sensitivity of 0.98 and PPV of 0.76.

Summary/Conclusions: To consider variant calling results in clinical routine, it does not seem appropriate to rely on the output of a single tool only. Instead, combining the output of several tools and applying a set of filters as it is done by our appre8 pipeline leads to results with both high sensitivity and PPV. Nonetheless, variant calling results should, especially at allelic frequencies below 20%, always be viewed with criticism.

E1198
COMPARISON OF ADMINISTRATION OF HYPOMETHYLATING AGENTS TO THE EFFICACY OF ALLOGENEIC SCT IN ELDERLY PATIENTS WITH ADVANCED MDS
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Background: Hypomethylating agents (HMA) have been introduced as a promising agent in the treatment of elderly patients with advanced myelodysplastic syndromes (MDS) leading to a response in approximately 50% of patients. However, most of the patients relapse and estimated years survival is below 10%. Stem cell transplantation (SCT) still represents the only curative treatment even in elderly patients with advanced MDS and it is connected with long-term survival in 35-40% despite relatively high risk of transplant related mortality (25-30%).

Aims: The aim of the study was a retrospective analysis of results of the treatment of 59 elderly patients (50 years of age or older) with MDS RAEB-2 or with acute myelogenous leukemia with multilineage dysplasia with less than 50% of bone marrow blasts (MDS RAEB-T according to the FAB classification) who received either HMA or underwent allogeneic SCT.

Methods: In the HMA group, 34 out of total 38 patients received azacitidine (Vidaza®) in the dose of 75mg/m²×7 every 28 days and 4 patients were treated with decitabine (Dacogen®) in the dose of 20mg/m²×5 every 28 days. Median number of cycles administered was 10.4 (range 3-31). An age and diagnosis matched transplanted group consisted of 21 patients, 9 patients were transplanted upfront, 12 patients were pretreated either with combination chemotherapy (10 patients) or with HMA (2 patients) and achieved CR prior to SCT. Ten patients received myeloablative conditioning and 11 patients were transplanted after reduced conditioning regimen.

Results: A hematologic response to HMA (CR,PR, hematologic improvement) was observed in 22 out of 38 patients in HMA group (57.9%), CR was achieved in 10 patients (31.8%). In SCT group, engraftment was reached in 20 out of 21 patients, 11 patients died after SCT (6 on complications related to SCT, 5 patients relapsed). No difference was observed between both the groups in 2 years estimated overall survival (OS), (42% for SCT vs 36% for HMA), a significant difference in favour of SCT was present in estimated 3 years and 5 years OS (42% and 38% vs SCT 9% and 4% in HMA group, P=0.001). Median OS was 18.7 months in HMA treated group compared to 42.6 months in SCT group (P=0.02). In a recent analysis performed at 48 months after starting the treatment, 2 patients treated with HMA (5.3%) and 9 patients treated with SCT (42.8%) were alive, 23 patients in HMA group and 6 patients in SCT group relapsed. No significant differences in results and adverse effects of treatment were observed between patients aged 50-60 years and those older than 60 years in both HMA and SCT groups.

Summary/Conclusions: Our results confirm previous observations showing that despite a promising effect of HMA resulting in hematologic response in more than 50% of elderly patients with advanced MDS, allogeneic SCT still represents the only potentially curative treatment connected with long-term survival in a significant number of patients even in elderly MDS patients.
Background: Rigosertib, a novel phosphoinositide 3 kinase pathway inhibitor, induces G2/M arrest leading to the apoptosis of cancer cells and myeloblasts and is safe for and well tolerated by pts with low, intermediate-1, intermediate-2, or high-risk myelodysplastic syndromes (MDS).

Aims: The aims of the study were to assess the safety, efficacy, and pharmacokinetics of oral rigosertib and to determine the recommended dose (RD) for a Phase II clinical study in Japanese pts with recurrent/relapsed or refractory MDS.

Methods: We conducted a multicenter, open-label, Phase I clinical study of oral rigosertib. The key eligibility criteria were as follows: recurrent/relapsed or refractory MDS; age ≥20 or older; ECOG PS of 0 to 2; and no major organ dysfunctions. Rigosertib (280 and 560mg BID) was administered orally in one 21-day cycle (up to cycle 6) that consisted of the 14-day, twice-daily, oral administration term, followed by 7-day monitoring. The primary endpoint was dose-limiting toxicity (DLT). The secondary endpoints were 1) safety as assessed with adverse events (AEs) and laboratory results, 2) efficacy as assessed with the International Working Group 2006 criteria, and 3) pharmacokinetics.

Results: Between March 2013 and November 2014, 6 male and 3 female pts (median age: 70; range 52-80) were enrolled. ECOG PS was 0 in 7 pts and was 1 in 2 pts, and 3 and 6 pts were eventually assigned to the 280 and 560mg BID arms, respectively. According to the FAB classification, 4, 2, 2, and 1 pts were categorized to RAEB, RARS, RA, and RAEB-1, respectively. The prognostic factor according to IPSS was Int-1 risk in 4 pts (1 and 3 pts in the 280 and 560mg BID arms, respectively) and was Int-2 in 5 pts (2 and 3 pts in the 280 and 560mg BID arms, respectively). DLT occurred in 1 pt in the 280mg BID arm and in 2 pts in the 560mg BID arm: the former consisted of type 2 diabetes and grade 4 delirium, and the latter grade 5 urinary tract infection and grade 3 prolonged QT interval. Therefore, the RD for a Phase II clinical study in Japanese pts was determined to be 560mg BID. On day 11 of treatment, 1 pt in the 560mg BID arm died of grade 5 urinary infection whose relationship with the investigational drug was rated to “Definite”. The presumed cause of death was a septic shock due to urinary tract infection. The mean counts of leukocytes, neutrophils, lymphocytes, and reticulocytes in the 280mg BID arm did not decrease along with increases in the number of cycles delivered but decreased slightly in the 560mg BID arm. Any changes of note were not found in other hematological items. One case of grade 3 neutropenia developed in the 280mg BID arm, and 1 case each of grade 3 laboratory abnormalities—increased alanine aminotransferase, increased aspartate aminotransferase, prolonged QT interval, neutropenia, and decreased hemoglobin—occurred in the 560 BID arm. The hematological remission rate was 11.1% (1 marrow CR, 1/9 pts), and the hematological improvement rate was 11.1% (1 HI-P: 1/9 pts). Among the PK parameters, inter-individual variability was observed in the Cmax and AUC. However, changes suggesting the accumulation of rigosertib during repeated oral administration (e.g., consistent increases in the Cmax and AUC) were not found.

Summary/Conclusions: The present chemotherapy regimen of oral rigosertib was well tolerated. Our study indicates that the RD for a Phase II clinical study is 560mg BID in Japanese patients with recurrent/relapsed or refractory MDS.

E1200
NON-OVERLAPPING PROMOTER AND SUPER-ENHANCER DRIVEN PROCESSES SUPPORT MYELOMA CELL GROWTH AND SURVIVAL VIA DISTINCT REGULATORY AXES
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Background: We have previously reported that E2F1 and its heterodimerization partner DP1 promote MM tumor proliferation both in vitro and in vivo; and observed an inverse correlation between their expression and patient survival suggesting a role in MM pathogenesis. Moreover, E2F functional impairment by a dimerization inhibiting stapled peptide significantly affects myeloma tumor cell growth while sparing effect on normal components of bone marrow as well as normal plasma cells, suggesting an E2F dependency in MM cells.

Aims: In this study, our aim was to defined the regulatory landscape of E2F in MM to better understand how E2F1 and DP1 drive myeloma cell proliferation; and to define the relationship between promoter proximal transcription factor-associated gene expression and super-enhancer-driven transcriptional programs.

Methods: We integrated genetic perturbation with functional omics to define E2F role in MM. Global occupancy of E2F1 and DP1 in MM was evaluated by ChIP-seq analysis. E2F1 and DP1 genomic localizations were then integrated to MM reference epigenome. Enhancers and super-enhancers were mapped using Rroad densities were calculated using bamliquidator (github.com/BradnerLab/pipeline/wiki/bamliquidator).

Results: Integration of E2F1 and DP1 genomic localization to MM reference epigenome revealed specific co-occupancy of the factors at promoters of active genes marked by H3K4me3, with a strong positive correlation between E2F and RNA Pol II increase at RNA Pol II binding at transcription start sites. In contrast, active enhancers, as defined by promoter distal Mediator (MED1) peaks and marked by H3K27ac and BRD4, showed virtually no E2F binding. Prompt by these observations, we explored the transcriptional and functional interrelationship between E2F and BETs to identify their individual contribution to eventual functional effect in MM. Unbiased hierarchical clustering revealed distinct regulatory axes for E2F and BETs, with E2F predominantly localized to active gene promoters of growth/proliferation genes and BETs disproportionately at enhancer-regulated tissue specific genes confirming that these factors establish distinct target gene programs. At the extremes, we found less than 10% of genes were among the top 500 in BRD4 enhancer signal (i.e. SE-regulated) and top 500 E2F promoter signal. We hypothesized that the presence of BETs and E2F in distinct regulatory axes divides active genes in MM into those that can be selectively influenced by E2F inhibition or E2F perturbation, but not both. In line with this we have observed that dual E2F and BET inhibition is synergistic for MM cell growth, both in vitro and in vivo.

Summary/Conclusions: In conclusions, our results highlight the existence of non-overlapping promoter and super-enhancer-associated dependencies in multiple myeloma, suggesting a sequestered molecular control that may be perturbed in cancer with potential for development of a promising therapeutic strategy.
Aims: We used a custom target pulldown (TPD) approach on a large cohort of MM patients at diagnosis, with homogeneous treatment and long follow-up, to further our understanding of the landscape of driver lesions in MM and how this can be used to improve prognostication and disease classification.

Methods: We used a custom-designed SureSelect pulldown strategy (Agilent Biotechnologies) to target 246 genes implicated in MM or cancer in general; 2538 single nucleotide polymorphisms; the immunoglobulin heavy chain (IGH) locus. We sequenced unmatched DNA from CD138-purified plasma cells from 418 patients with a median follow-up of 5.4 years using Illumina Hiseq2000 machines. We applied algorithms developed in-house to detect driver genomic events, filtering out potential artifacts and germline variants. We then ranked each mutation on its likelihood of being oncogenic.

Results: We identified 197 driver events including gene mutations, aneuploidies and IGH translocations (IGH-Tx), median of 6 per patient. Gene mutations where found in >99% of patients. At least one oncogenic mutation of a known driver gene previously identified (KRAS, NRAS, TP53, FAMM6C, BRAF, DIS3, TPAF1, SPH40, RRF4) was found in 64%, with a long tail of infrequently mutated genes with uncertain significance. Karyotypic class was assigned in 80% of patients, with 9% of hyperdiploid cases also showing an IGH-Tx (mostly t(4;14)). IGH-Tx and aneuploidies dominated the MM genomic landscape, KRAS and NRAS being the only point mutations present in the 15 most frequent driver events. Multivariate analysis by sparse Cox regression highlighted only four driver events with significant prognostic impact for both progression-free (PFS) and overall survival (OS): t(4;14) (HR 1.88, CI 1.25–1.84), amp(1q) (HR 2.63, CI 1.92–3.59), del(17p) (HR 2.55, CI 1.66–3.92), and rare mutations of ATP13A4 (HR 0.08, CI 0.01–0.65, mutated in 1.4% of patients). We found a significantly worse prognosis for increasing numbers of driver lesions in each patient (median OS 8.2 vs 3.5 years for <5 and >8 driver events, respectively). This was only partially explained by instances of additive effect or interactions between variables, which were very informative but not frequent. To better investigate these findings in the context of the genomic landscape of each case, we applied Bayesian clustering algorithms. The large number of driver events screened led to the identification of three groups: in the largest group, some hyperdiploid and IGH-Tx cases clustered together, suggesting that secondary mutations and CNAs required for tumor progression are often shared between these two subgroups. We then identified two clusters both characterized by significantly lower number of mutations, but with opposing features. One was enriched for IGH-Tx, had the highest number of CNAs overall, showed higher prevalence of amp(1q), del(13), del(17p), TP53 mutations, and had a shorter median OS of 5.3 years. The other was mostly composed of hyperdiploid cases and showed fewest CNAs and mutations, with a good prognosis (median OS not reached).

Summary/Conclusions: We report on the first attempt towards the use of extended tumor genotype for a genomic classification of MM using innovative clustering algorithms. Despite the heterogeneity of the disease, we could identify disease subgroups with a distinct spectrum and number of driver events carrying different prognosis, supporting the introduction of genomics in the clinical approach to MM.

E1202
A NOVEL METHOD FOR GENOME-WIDE COPY NUMBER ASSESSMENT FROM TARGETED SEQUENCING DATA AND CLINICAL APPLICATION IN PATIENTS WITH MULTIPLE MYELOA

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Background: Assessment of gene mutations by next generation sequencing is now standard in patients with haematological malignancy. However, larger chromosomal aberrations (e.g. exon, gene and chromosome level gains and losses) also serve as critical prognostic indicators that guide therapeutic decision making. These larger gene lesions are typically assessed using a separate methodology such as conventional cytogenetics/FISH.

Aims: We aimed to develop and clinically validate a novel method for assessing genome-wide copy number changes using an existing hybridisation-based targeted sequencing panel in order to further critical prognostic information from large-scale data without the need for a separate assay.

Methods: A custom Agilent SureSelect capture panel targeting 313 genes of relevance in myeloid and lymphoid malignancies was sequenced on an Illumina NextSeq (paired end 75bp reads) to a mean depth of 700x. An in-house bioinformatics pipeline was created to analyse probe counts from on-target and off-target regions which also included CNAs and ON-target and sequencing by normalisation to a pooled reference comprising 10 normal controls. Three metrics for copy number calling were tested including a permutation-based statistic from circular binary segmentation, weighted mean and variance for the bins in each segmented region, and an MLPA-like test using reference data. Vizualisation tools compared to controls. An interactive web-based graphical user interface was developed to visualise both large-scale and exon level amplification and deletions.

Results: We validated the approach on 45 samples from patients with multiple myeloma (predominantly advanced disease) with known copy number status as determined by conventional cytogenetics, FISH and MLPA. Our novel method detected numerous copy number changes that were outside the targeted region (through genome-wide mapping and analysis of off-target reads) such as del(1p) in 12 patients, gain(1q) in 15 patients and MYC amplification in 5 patients. Moreover our method was able to interrogate and resolve the complexity of changes on del(1p) including isolated deletions of FAMM6C, CDKN2C and FAP1. Of 25 patients with a TP53 mutation, 20 had concomitant del(17p) detected by our assay, while 1 case had a del(17p) without mutation; both monosomy and biallelic TP53 aberration was associated with poor survival. Other findings in this cohort include frequent DI3 mutations in patients with refractory disease and >13 and >17 copies of chromosome 3. Mutations in the entire panel were found in >99% of patients. At least one oncogenic mutation of a known driver gene was found in >99% of patients. The availability of multiple sets of high quality genomic data associated with haematological malignancy. In the context of myeloma this can be used to report clinically relevant changes including deletions of 1p and 17p, and gains of 1q and 8q, as well as novel numerical chromosome aberrations.

Summary/Conclusions: We have developed and demonstrated utility of a reliable workflow for genome-wide copy number assessment that can be implemented using existing targeted short read sequencing data, greatly extending the utility of this technology beyond the identification of alterations for patients with haematological malignancy. In the context of myeloma this can be used to report clinically relevant changes including deletions of 1p and 17p, and gains of 1q and 8q, as well as novel numerical chromosome aberrations.
80% power to detect gene expression changes and genomic variants associated in >2% of the study population. WES data identified the main cytogenetic groups, somatic variants, and significantly mutated genes. 28 significantly mutated genes were present in newly diagnosed samples (17 genes in >2% of samples). The main recurrent mutations included KRAS and NRAS, and negative regulators of the NF-κB pathway; however, novel genes were also identified. One distinct mutational patterns, proportions, and sites between translational subgroups were found and will be presented. In addition, we detected recurrent copy number abnormalities and examined the interaction with mutations and fusion gene expression from RNASeq. Preliminary analysis with an integrative model developed with machine learning methods/approaches using CN, SNV and structural variants predicted a subset of high-risk patients. Unsupervised molecular classification is in progress to integrate genomic data and define subgroups, which will be presented.

**Summary/Conclusions:** We have established the largest repository of molecular profiling data in MM associated with clinical outcomes. Integrated analyses are enabling generation of clinically meaningful disease segments associated with differing risk that will inform development of clinical tests. ThempP intends to build a global network by expanding collaboration with global MM centers to incorporate additional datasets through current and new collaborations.

*The first 6 authors share co-first authorship. The last 3 authors share co-senior authorship.

**E1204**

**ALVOCIDIB SYNERGIZES WITH VENETOCLAX IN PRECLINICAL MODELS OF MULTIPLE MYELOMA**

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**Background:** With over 30,000 new cases expected in 2016 (US), new treatments are desperately needed for the treatment of multiple myeloma (MM). Major developments in the treatment of MM have included introduction of agents such as lenalidomide, thalidomide, or bortezomib. Bortezomib, an inhibitor of the proteasome, reduces the degradation of many proteins, including the pro-apoptotic protein NOXA. However, high levels of MCL1 and/or low basal levels of NOXA have been implicated in bortezomib resistance and negative patient outcomes. The BCL-2-specific BH3 mimetic, venetoclax (ABT-199), is also being explored in multiple hematologic malignancies, including multiple myeloma. However, intrinsic resistance to venetoclax treatment observed in MM patient samples has been attributed to a low BCL-2 to MCL1 gene expression ratio, suggesting a central role for MCL1 in cell survival in this context as well. NOXA functions to sequester the anti-apoptotic BCL-2 family member, MCL1. Increased MCL1 expression is a known resistance mechanism to venetoclax treatment in a variety of cell types including chronic lymphocytic leukemia and lymphomas. Considering the central role of MCL1 to treatment efficacy in MM, we investigated the ability of an MCL1-lowering agent, namely the CDK9 inhibitor alvocidib, to potentiate the activity of venetoclax in MM. Alvocidib suppresses MCL1 expression via CDK9-mediated regulation of RNA polymerase II. Alvocidib has demonstrated robust improvements in the in vivo TNFα signaling, and unfold protein response, were activated by OSSL_325096. Indeed, in the cell-free ATPase assay, OSSL_325096 showed dose-dependent inhibition of VCP’s ATPase activity (Figure 3). The IC50 of OSSL_325096 on ATPase activity was 7-10µM, while IC50 of cell survival in MM cells was 0.1-0.8µM, suggesting that OSSL_325096 may have other anti-myeloma function besides VCP inhibition by OSSL_325096. For example, OSSL_325096 twice a week.

**Results:**

VCP inhibition by OSSL_325096. For example, OSSL_325096 twice a week.

**Summary/Conclusions:** The present data suggest that OSSL_325096 may be novel anti-myeloma drug candidate partially through its direct inhibition activity of VCP.

**E1206**

**A NOVEL PREDICTIVE MODEL COMBINING LINCRNAS AND PROTEIN CODING GENES IN MULTIPLE MYELOMA**

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**Background:** VCP (p97) is an ER-associated protein that belongs to the AAA ATPase family. It has a variety of cellular functions including ER-associated protein degradation, autophagy, and aggresome formation. Recent studies have elucidated emerging roles of VCP and its potential as a therapeutic target in several cancer subtypes including multiple myeloma (MM). Aims: We screened approximately 2,000 small molecular compounds to find out novel small compounds that suppress growth of MM cell lines, and found that OSSL_325096 has strong anti-proliferative activity on MM cell lines (IC50 100-500µM). In this study, we evaluated anti-MM activity of OSSL_325096 through VCP inhibition, in an ATP-competitive manner.

**Methods:** OSSL_325096 were purchased from Princeton BioMolecular Research, Inc. (Princeton, NJ, USA). His-tagged human VCP (hVCP) cDNA was cloned and utilized to generate hVCP protein in vitro as previously described (Chou et al. PNAS, 2011, vol. 108(12): 4834-4839) to evaluate the VCP inhibition by OSSL_325096. For in vivo analysis, MM xenograft model mice were intraperitoneally administered with vehicle or 50mg/kg of OSSL_325096 twice a week.

**Results:** OSSL_325096 inhibited proliferation of MM cell lines, including one bortezomib-resistant cell line (Figure 1). OSSL_325096 induces apoptosis in these MM cell lines and primary MM cells purified from patients but not in PBM-Cs from healthy donors. OSSL_325096 treatment leads to accumulation of poly-ubiquitinated proteins, cleavage of caspase-3, and up-regulation of CHOP in MM cell lines (Figure 2), suggesting this compound induces caspase-mediated apoptosis and ER-stress in MM cells. OSSL_325096 has a chemical structure similar to several known VCP inhibitors. Therefore, to evaluate the role of VCP in MM cell lines, we next performed knockdown of VCP. Knock-down of VCP induced apoptosis in MM cell lines, accompanied with accumulation of poly-ubiquitinated protein. In-silico protein-drug binding simulation suggests possible binding of OSSL_325096 to the ATP binding site in VCP’s D2 domain. Indeed, in the cell-free ATPase assay, OSSL_325096 showed dose-dependent inhibition of VCP’s ATPase activity (Figure 3). The IC50 of OSSL_325096 on ATPase activity was 7-10µM, while IC50 of cell survival in MM cells was 0.1-0.8µM, suggesting that OSSL_325096 may have other anti-myeloma function in addition to VCP inhibition. RNA-sequencing of MM cells treated with OSSL_325096 revealed that several pathways including mTORC1 signaling, TNFα signaling, and unfold protein response, were activated by OSSL_325096. Finally, OSSL_325096 was administered to xenograft mice with MM cell tumors and inhibited the tumor growth in vivo (Figure 4).
Background: RNA has diverse sets of regulatory functions and a recent analysis of a human repertoire has identified a large number of non-coding transcripts. One of which, long intergenic non-coding RNA (lincRNA) with transcripts longer than 200 nucleotides, are located between the protein coding genes and do not overlap exons of either protein-coding or other non-lincRNA genes. lincRNAs have been considered to provide regulatory functions, however, their precise functions remain unclear. In the present study, we aimed to investigate the nature of both exons of either protein-coding or other non-lincRNA genes. lincRNA data.

Results: Using only the expressed lincRNA, we developed a risk prediction signature by using Spearman’s rank estimates of EFS at 4 years were 55% (95% CI, 45.1% to 63.1%) and 32% (95% CI, 25.1% to 42.2%), and OS at 4 years were 93.2% (95% CI, 88.9% to 97.6%) and 71.1% (95% CI, 62.9% to 80.3%) in our patients having a low or high risk score. We then combined lincRNA signature with known expression signatures and improved the risk prediction for known expression signatures dramatically. We validated our results on independent large cohort with newly diagnosed MM RNAseq data. When applied to patient cohort separated by other risk categorization including minimal residual disease status (MRD), cytogenetic status risk (del17p, t(4;14) and t(14;16)) and International Staging System (ISS), lincRNA signature was able to further identify patients with significant differential survival outcomes.

Summary/Conclusions: In summary, we report that lincRNAs have an independent effect on survival outcome in MM and provides rationale for its use in risk stratification as well as to understand biological impact. Combined risk prediction with other risk features improve the prediction power and helps to create better classification in MM.

**E1207**

**DYNAMIC IMMUNOHISTOCHEMICAL EVALUATION OF MARROW MICROENVIRONMENT MODIFICATIONS IN PATIENTS WITH SMOLDERING MYELOMA**

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Background: In most cases, multiple myeloma (MM) is preceded by an asymptomatic status known as monoclonal gamopathy of unknown significance (MGUS) or smoldering multiple myeloma (SMM). The mechanisms of progression from SMM to MM are not well understood. Despite an increasing evidence of an immune system dysregulation in the setting of MM characterized by a tolerant microenvironment, the immunosurveillance profile in the setting of SMM has never been investigated.

Aims: Our aim was to identify a progressive dysregulation of the immune microenvironment in patients with post SMM versus those with stable SMM.

Methods: We performed extensive immunohistochemical analysis of bone marrow samples of 16 patients affected by SMM at time 0 (16 samples) and at +24 months (+/- 4 months, 16 samples). Half of these patients developed MM at 24 months (progressed SMM), the other half remained asymptomatic (stable SMM). Immunohistochemical analysis comprised the following markers: microenvironment components CD38, CD4, CD8, CD3, CD45, CD56, CD68, loss of immunogenicity (PD-L1, PD-L2, PD-1, LAG3, CTLA-4, IDO), loss of antigenicity (HLA-DR), Immunogenicity and antigenicity markers expression was described as percentage on the total of marrow plasma cells and non-plasma cells separately. A first analysis compared the samples of the whole cohort at time 0 (16 samples) and the subgroups with residual disease (CD4+ vs. CD8+, CD4+/CD8+ ratio) and with residual disease in patients at 12 months post ASCT. Patients with residual disease (RD) had higher levels of cytotoxic (Foxp-3+) CD8+ effectors expressing GzmB, which were not significantly higher compared to healthy donors.

Results: At time 0, we found an increased plasma cell marrow infiltration in the progressed SMM group (28% vs. 23%, p=0.01). At time +24 months, no differences were observed but an increased plasma cell marrow infiltration in the progressed SMM group (50% vs. 26%, p=0.01).

Summary/Conclusions: First, we observed an increase in inflamed microenvironment markers (increase in CD4+ and CD8+ cells) in favor of CD4+ population and HLA-DR expression on plasmacytomas and non-plasmacytomas during the course of SMM. Second, expression of T cell inhibition markers (PD1, LAG3) was significantly augmented during disease progression. For the first time, we reported a comprehensive analysis of microenvironment modifications in untreated samples and we observed an immune dysregulated microenvironment were observed. In particular, increased in PDL1 and LAG3 expression could be of clinical interest due to the current availability of checkpoint inhibitors drugs targeting these molecules. The results of this study should be confirmed on prospective studies with larger number of patients.

**E1208**

**IMMUNE CELL PROFILING IN BONE MARROW OF MYELOMA PATIENTS POST AUTOLOGOUS STEM CELL TRANSPLANTATION SHOWS PRESENCE OF CYTOTOXIC CD4 AND CD8 CELLS, WITH PROMINENT LAG-3 EXPRESSION AND OTHER CHECKPOINT MARKERS**


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Background: Multiple myeloma (MM) is a plasma cell malignancy that remains incurable, despite therapeutic advances. Immunotherapies have recently shown much promise in this and other cancers, and are under intense investigation. Autologous stem cell transplantation (ASCT) is standard of care in young fit newly diagnosed patients. In the post-ASCT setting, the minimal disease burden and re-constituting immune system may be a favourable context for immunotherapies, including cellular therapy and checkpoint blockade. Autologous cells aimed to challenge the immune system (BM) of myeloma patients post-ASCT, to identify candidate immune checkpoint proteins for therapeutic targeting.

Methods: BM aspirates were obtained from patients with MM at 3months post ASCT (n=28), and 6-12months post ASCT (n=41) at University College Hosp.

Aims: CD8+ cells exceeded CD4+ early as 3months post-ASCT, suggesting the BM compartment is rapidly filled with CD8 cells. Although absolute numbers of CD4 effectors (CD4+Foxp3-) were either similar (3 months) or to lower (6-12months, p<0.05) than healthy donors, there was a higher proportion of cytotoxic (GzmB+) CD4 cells (3months median 30.4%, range 0.2-89.7%, p<0.01 vs control median 2.6%, range 0.2-33.4% and 6-12months, median17.6%, range 0.4-100%, p<0.05). CD4 effectors also expressed activation markers: Inducible co-stimulator (ICOS, 3months median 20.2%, range 2.9-80.6%, p<0.05 vs control median 8.6%, range 2.2-24% and 6-12months median 33%, range 1.8-80.7%, p<0.01 and HLA-DR (p<0.05), which was directly associated with the proportion of lymphocytes (CD8+CD45RA-CD62L-CD27-) as well as increased levels of GzmB and LAG3, which were not significantly higher compared to healthy donors.

Results: At time 0, we found an increased plasma cell marrow infiltration in the progressed SMM group (28% vs. 23%, p=0.01). At time +24 months, no differences were observed but an increased plasma cell marrow infiltration in the progressed SMM group (50% vs. 26%, p=0.01).

Summary/Conclusions: First, we observed an increase in inflamed microenvironment markers (increase in CD4+ and CD8+ cells) in favor of CD4+ population and HLA-DR expression on plasmacytomas and non-plasmacytomas during the course of SMM. Second, expression of T cell inhibition markers (PD1, LAG3) was significantly augmented during disease progression. For the first time, we reported a comprehensive analysis of microenvironment modifications in untreated samples and we observed an immune dysregulated microenvironment were observed. In particular, increased in PDL1 and LAG3 expression could be of clinical interest due to the current availability of checkpoint inhibitors drugs targeting these molecules. The results of this study should be confirmed on prospective studies with larger number of patients.

**E1209**

**INHIBITION OF EXTRACELLULAR VESICLE SECRETION INDUCES APOPTOSIS OF BONE MARROW STROMAL CELLS: TOWARDS SOIL-TARGETED THERAPY IN MULTIPLE MYELOMA**

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Background: Around 10% of the patients with monoclonal gammopathy of unknown significance (MGUS) or smoldering multiple myeloma (SMM) will transform into multiple myeloma (MM). Progression from SMM to MM is poorly understood. Despite an increasing evidence of an immune system dysregulation in the setting of MM characterized by a tolerant microenvironment, the immunosurveillance profile in the setting of SMM has never been investigated.

Aims: Here, we have studied lincRNAs using uniformly treated patients to show their impact on survival outcome in MM.

Methods: We performed RNA-seq on CD138+ MM cells from 360 newly-diagnosed patients and 18 normal plasma cells (NPM) and analyzed for lincRNA and protein coding genes. We performed extensive immunohistochemical analysis of bone marrow with residual disease (CD4+ vs. CD8+, CD4+/CD8+ ratio) and with residual disease in patients at 12 months post ASCT. Patients with residual disease (RD) had higher levels of cytotoxic (Foxp-3+) CD8+ effectors expressing GzmB, which were not significantly higher compared to healthy donors.

Results: At time 0, we found an increased plasma cell marrow infiltration in the progressed SMM group (28% vs. 23%, p=0.01). At time +24 months, no differences were observed but an increased plasma cell marrow infiltration in the progressed SMM group (50% vs. 26%, p=0.01).

Summary/Conclusions: First, we observed an increase in inflamed microenvironment markers (increase in CD4+ and CD8+ cells) in favor of CD4+ population and HLA-DR expression on plasmacytomas and non-plasmacytomas during the course of SMM. Second, expression of T cell inhibition markers (PD1, LAG3) was significantly augmented during disease progression. For the first time, we reported a comprehensive analysis of microenvironment modifications in untreated samples and we observed an immune dysregulated microenvironment were observed. In particular, increased in PDL1 and LAG3 expression could be of clinical interest due to the current availability of checkpoint inhibitors drugs targeting these molecules. The results of this study should be confirmed on prospective studies with larger number of patients.
Background: Bone marrow stromal cells (BMSCs) interact with multiple myeloma (MM) cells in the bone marrow, and also create a permissive microenvironment for MM cell growth and survival. Recent evidence indicated that MM cell-BMSC communication via extracellular vesicles (EVs) plays an important role in the MM microenvironment.

Aims: In this study, we investigated the biological property of EVs and miRNAs in EVs derived from BMSCs, aiming to establish the emerging strategies to target MM microenvironment to prevent tumor growth and spread.

Methods: Bone marrow samples were obtained from MM patients (age 56 to 82, n=20) and monoclonal gammopathy of undetermined significance (MGUS) patients (age 44 to 82, n=13) in accordance with the Declaration of Helsinki and using protocols approved by the research Ethics Committee of Tokyo Medical University (IRB No. 2648), and BMSCs derived from MM patients (MM-BMSCs) and MGUS patients (MGUS-BMSCs) were isolated by the classical adherence method. EVs were isolated from conditioned medium of BMSCs using a Exoquick-TC (SBI). The size of EVs was confirmed using a NanoSight LM10 (Malvern). The RNA from cells and EVs was profiled for 381 miRNAs using a TaqMan low-density array (ABI). Cell viability of miRNA-overexpressed BMSCs were determined using WST-8 (Dojindo), and Apoptosis rates were determined using Caspase-Glo assays (Promega). To assess the effect of the inhibitor on EV secretion, BMSCs were treated with 10 µM GW4869 (nSMase2 inhibitor, Sigma) for 48h. Rested MM-BMSCs and MGUS-BMSCs had a fibroblast-like morphology in culture, and were homogeneously CD73+, CD90+, CD105+, CD34-, and CD45-. MM-BMSCs had a higher expression of α-smooth muscle actin (α-SMA) than MGUS-BMSCs. The nanoparticle size distribution of EVs derived from BMSCs was approximately 50 nm. We found high expression of miR-10a in the EVs derived from MM-BMSCs, while the expression of intracellular miR-10a was low in MM-BMSCs. We therefore hypothesized that low expression of cellular miR-10a might be important for survival of MM-BMSCs; As a result, miR-10a was packaged into EVs, and they were released to the extracellular space. To test the hypothesis, miR-10a mimic was transfected into MM-BMSCs and MGUS-BMSCs. We found that overexpression of miR-10a inhibited cell proliferation and induced apoptosis of MM-BMSCs, while the cell proliferation and apoptosis of MGUS-BMSCs were not affected by the overexpression of miR-10a. We also found that inhibition of EV release with GW4869 promote the accumulation of intracellular miR-10a in MM-BMSCs, and EV release inhibitor also can inhibited cell proliferation and induced apoptosis of MM-BMSCs.

Summary/Conclusions: Our results provide the possibility that the inhibition of EV secretion induced apoptosis of MM-BMSCs that can support MM cell growth and survival in BM microenvironment.

E1210
SINGLE-NUCLEOTIDE POLYMORPHISM IN THE PBK GENE IS CLOSELY ASSOCIATED WITH MYELOMA CELL PROLIFERATION
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Background: Elevated expression of the PDZ binding kinase (PBK), which encodes a serine/threonine kinase, has been reported to be associated with a poor prognosis in a variety of cancers. The public gene expression profiling data also showed that higher expression of PBK was related with a poor prognosis in myeloma. However, the molecular mechanisms of PBK gene functions associated with myeloma cell growth and survival in vivo and in vitro have never been investigated in myeloma.

Aims: The aim of this study was to elucidate PBK gene functions associated with myeloma cell growth in vitro and in vivo.

Methods: Eight human myeloma cell lines including ANBL-6, 8226, OPM2, and KMS-11 were used in this study. The expression levels of miRNA and protein of PBK were detected by real-time RT-PCR and western blotting, respectively. The genome sequence of the whole PBK gene was determined using the dye terminator method. Knockout of PBK was performed using CRISPR-Cas 9 system. A single guide RNA sequence for PBK was in exon 3 and PBK expression was completely disrupted (Fig. 1). Transfection of the plasmid expressing PBK to cells was performed using with the Amaxa Nucleofector system. Cell viability and proliferation were examined by the MTT and colony formation assay. The KMS-11 cells were subcutaneously injected to mice and tumor volumes were observed every 3 to 4 days.

Results: High expression of mRNA and protein of PBK was observed in 8/8 myeloma cell lines. Genome sequencing revealed the rs3779620 polymorphism in the 5’UTR of PBK gene. In the mouse model, the A to G transition results in the N107S substitution. A/A, A/G, and G/G were found in 88, 0, and 12%, respectively. Of note, PBK inhibition by CRISPR-mediated knockout enhanced cell proliferation in ANBL-6, 8226, and OPM2 cells, all of which carry PBK G/G. Surprisingly, in the KMS-11 cells carrying PBK G/G, PBK inhibition by CRISPR-mediated knockout suppressed cell growth in vitro and in xenograft mice (Fig. 2). Moreover, exogenous expression of PBK G/G augmented cell proliferation in the PBK-deficient OPM2 cells, which carry PBK A/A. Furthermore, Thr 9 phosphorylation on PBK was increased in cells expressing PBK G/G compared with those cells expressing PBK A/A.

Summary/Conclusions: Our findings indicate that expression of PBK G/G was associated with myeloma cell proliferation, while PBK A/A was likely linked to tumor suppression. Increased phosphorylation of Thr 9 on PBK might contribute to proliferation in cells with PBK G/G. These results provide a novel insight into the mechanisms underlying myeloma cell growth and PBK rs3779620 genotype is a potential stratification and therapeutic target for plasma cell dyscrasias.

E1211
THE HISTONE METHYLTRANSFERASES G9A/GLP REPRESENT NEW PROMISING TARGETS FOR THE TREATMENT OF MULTIPLE MYELOMA
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Background: Multiple myeloma (MM) is a clonal plasma cell malignancy which mainly resides in the bone marrow. In cancer cells, the epigenetic landscape is known to be highly disturbed. In line, numerous epigenetic aberrations have been described in MM, resulting in deregulated gene expression, disease progression and drug resistance. Targeting deregulated epigenetic modifiers therefore represents an interesting therapeutic approach. G9a (EHMT2) and GLP (EHMT1) are 2 histone methyltransferases which catalyze mono- and dimethylation of histone H3 lysine 9 (H3K9). Importantly, G9a is overexpressed in several cancers, correlating with a poor prognosis.

Aims: Currently, data about the expression and role of G9a/GLP in MM is lacking. The aim of this study is therefore to investigate the functional role of G9a/GLP in MM pathogenesis.

Methods: The prognostic value of G9a/GLP in terms of overall survival was determined in the UAMS-TT2 cohort of newly diagnosed MM patients (n=345), using Cox proportional hazards regression analysis. In addition, we used a panel of 10 human cell lines, 3 murine cell lines and 5 primary patient samples to evaluate the effect of the small molecule inhibitors UNC0638 and BIX01294 on MM cell viability, cell cycle progression and apoptosis. We also assessed the in vitro anti-MM activity of BIX01294 in combination with bortezomib or ABT-199. The in vivo anti-MM activity of therapeutic BIX01294 treatment was tested using the murine STGEM1 model. Differences in overall survival between groups was determined in the UAMS-TT2 cohort of newly diagnosed MM patients (n=345, using a log-rank test and survival curves plotted using the Kaplan-Meier method).
Results: Here we report that high expression levels of both G9a and GLP are associated with a worse disease outcome in newly diagnosed MM patients. Moreover, gene set enrichment analysis of patients with high G9a/GLP expression levels displayed a significant enrichment of genes involved in pathways associated with MM disease progression, including the RAS pathway, NF-kB canonical pathway, IRF4 multiple myeloma program and mRNA splicing. Next, we treated specific G9a/GLP inhibitors BIX01294 and UNC1958 significantly and potently reduced MM cell viability in vitro. Moreover, both inhibitors also induce cell cycle arrest and apoptosis. When comparing between both inhibitors, BIX01294 was found to be the most potent in inducing apoptosis. Mechanistic studies for BIX01294 furthermore indicated that BIX01294 treatment mediates apoptosis through the up-regulation of multiple pro-apoptotic regulators, 5 cytokines, and markers of proliferation and degranulation across multiple lymphocyte subsets. Samples were stimulated with CD3 and CD28 to assess functional capacity. Dimensionality reducing clustering algorithmic analysis was used alongside traditional data analysis techniques to identify potential therapeutic opportunities.

Summary/Conclusions: Altogether, our results demonstrate for the first time the importance of the histone methyltransferases G9a/GLP in MM pathogenesis. Furthermore, specific targeting of G9a/GLP induces MM cell apoptosis, enhances MM sensitivity to ABT-199 and bortezomib and significantly delays tumor progression in the murine 5TM1 model. Thus, G9a/GLP targeting represents a promising strategy to improve treatment of MM.

E1213
P53-RESTORING SMALL MOLECULE CP-31398 INDUCES APOPTOSIS VIA INDUCTION OF REACTIVE OXIDATIVE SPECIES IN HUMAN MULTIPLE MYELOMA
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Background: Reactive oxygen species (ROS) are normal byproducts of a wide variety of cellular processes. ROS have dual functional roles in cancer cell pathophysiology. At low to moderate levels, ROS act as signal transducers to activate cell proliferation, migration, invasion, and angiogenesis. In contrast, high levels of ROS induce cell death. In multiple myeloma (MM), ROS overproduction is the trigger for apoptosis induced by several anticancer compounds, including proteasome inhibitors. However, no drugs that mainly affect oxidative stress are currently used for treatment of MM in the clinic. In MM, p53 status is an independent prognostic marker, since patients harboring p53 abnormalities are highly resistant to standard therapies, and the incidence of p53 mutations and deletions increases during disease progression. Therefore, restoration of p53 is an attractive strategy for treating advanced relapsed and refractory MM (RRMM) patients. CP-31398 (CP) is a small molecule that activates wild-type p53 or restores tumor-associated p53 mutants to wild type p53 function in multiple human cancer cell lines; this leads to cell cycle arrest and/or apoptosis. The growth of rhabdomyosarcoma cell lines can be inhibited by p53-dependent induction of ROS, but it is not clear whether CP-induced cytotoxicity proceeds via a similar pathway.

Aims: Our study was aimed at evaluating the anti-myeloma activity of CP.

Methods: MM cell lines (MM1S, RPMI8226, U266, KSMS, OPM2, Delta47, KMS11) and three primary patient samples were treated for 48 h. Consequently, the inhibitory effect of CP on MM cell line growth was assessed using a WST-1 assay. In order to elucidate the cytotoxic mechanism of CP, immunoblotting and flow cytometry analysis were performed. Measurements of cytosolic and mitochondrial ROS were performed with CellROX Deep Red or MitoSOX Red. For quantification of ROS, cells were analyzed by flow cytometry and fluorescence microcopy. The therapeutic potential of CP was evaluated by its ability to suppress tumor growth in vivo using the subcutaneous RPMI8226 murine xenograft model for human MM.

Results: In this study, we have demonstrated that the p53-activating small molecule CP-31398 effectively inhibited the growth of MM cell lines and primary MM cells. CP-31398 overproduction is the trigger for apoptosis induced by several anticancer compounds, including proteasome inhibitors. However, no drugs that mainly affect oxidative stress are currently used for treatment of MM in the clinic. In MM, p53 status is an independent prognostic marker, since patients harboring p53 abnormalities are highly resistant to standard therapies, and the incidence of p53 mutations and deletions increases during disease progression. Therefore, restoration of p53 is an attractive strategy for treating advanced relapsed and refractory MM (RRMM) patients. CP-31398 (CP) is a small molecule that activates wild-type p53 or restores tumor-associated p53 mutants to wild type p53 function in multiple human cancer cell lines; this leads to cell cycle arrest and/or apoptosis. The growth of rhabdomyosarcoma cell lines can be inhibited by p53-dependent induction of ROS, but it is not clear whether CP-induced cytotoxicity proceeds via a similar pathway.

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Results: Only N educated by SMM- and MM-MSC (both from patients at diagnosis, relapsed and refractory) significantly up-regulated Arg1, NOS2 and TNFα and exhibited suppressive effect with a reduction of T cell proliferation (p<0.001). By co-culturing educated-N with Human Brain Microvascular Endothelial Cells (HBMEC), we observed increased both tube length and number of branch points only in conditions where HBMEC were incubated with MM-MSC or MM-MSC-N (p<0.05). Adding Bortezomib, Lenalidomide or Pomalidomide during co-culture of PBMC with MM-MSC, isolated N showed a significant reduction of pro-angiogenic activity but did not lose immunosuppressive activity. To examine if PC play a role in MSC “activation”, before performing co-cultures with PBMC, we pre-treated HS-5 or HC-SCM with MM cell lines, PC pre-treatment drives a healthy MSC to activate N in immunosuppressive and pro-angiogenic cells. Implanting of mixtures of fluorescently labeled MM cells and healthy- or MM-MSC into zebrafish, animals coinfected with PC and MM-MSC showed enhanced tumor colonization and growth compared with those injected with PC and healthy MSC.

SUMMARY: The tumor microenvironment transformation frommGUS to MM is associated with progressive activation of MSC that have a pro-tumoral activity. Indeed SMM- and MM-MSC polarize N in immunosuppressive and pro-angiogenic N (NZ) in vitro. In addition, MM-MSC facilitate MM growth in vivo confirming their central role in tumor progression.

E1215
LONG TERM CR MULTIPLE MYELOMA PATIENTS STUDIED WITH NEXT GENERATION FLOW SHOW PREDOMINANTLY CURED VSmgUS-LIKE MINIMAL RESIDUAL DISEASE PATTERNS: A STUDY OF THE GTMM-TUSCAN GROUP FOR MULTIPLE MYELOMA
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Background: CR is a prerequisite for long term responses, progression free survivals, and ultimately overall survivals and cure. In the era of novel agents, many MM patients can achieve stringent CR (sCR), i.e. disease disappearance at serological, immunostaining level plus negativity of light chains (FLC). On the other hand most of these patients still will relapse and minimal residual disease (MRD) detection will play a crucial role in the very next future. Recently, two 8 colours tubes panel developed by the EuroFlow Consortium can detect MRD with an increased sensitivity and can be applied as standardized method to study multiple myeloma (MM) patients.

Aims: While many studies have looked at MRD status sequentially and soon after autologous or allogeneic stem cell transplantation with flow or molecular techniques, little is known about long term remission patients (>5-10 years) and in particular if more sensitive techniques such as NGS or NGS can still detect minimal disease in those patients. Aim of the study was to analyse patients with MM in >VGPR with next generation flow at >2 and >5 years of last remission.

Methods: Clinical assessment definition of CR status included serum and urine immunofixation, free light chain determination, imaging study with CT-PET, and in particular if more sensitive techniques such as NGS or NGS can still detect minimal disease in those patients. Aim of the study was to analyse patients with MM in >VGPR with next generation flow at >2 and >5 years of last remission.

Results: Only N educated by SMM- and MM-MSC (both from patients at diagnosis, relapsed and refractory) significantly up-regulated Arg1, NOS2 and TNFα and exhibited suppressive effect with a reduction of T cell proliferation (p<0.001). By co-culturing educated-N with Human Brain Microvascular Endothelial Cells (HBMEC), we observed increased both tube length and number of branch points only in conditions where HBMEC were incubated with MM-MSC or MM-MSC-N (p<0.05). Adding Bortezomib, Lenalidomide or Pomalidomide during co-culture of PBMC with MM-MSC, isolated N showed a significant reduction of pro-angiogenic activity but did not lose immunosuppressive activity. To examine if PC play a role in MSC “activation”, before performing co-cultures with PBMC, we pre-treated HS-5 or HC-SCM with MM cell lines, PC pre-treatment drives a healthy MSC to activate N in immunosuppressive and pro-angiogenic cells. Implanting of mixtures of fluorescently labeled MM cells and healthy- or MM-MSC into zebrafish, animals coinfected with PC and MM-MSC showed enhanced tumor colonization and growth compared with those injected with PC and healthy MSC.

SUMMARY: The tumor microenvironment transformation frommGUS to MM is associated with progressive activation of MSC that have a pro-tumoral activity. Indeed SMM- and MM-MSC polarize N in immunosuppressive and pro-angiogenic N (NZ) in vitro. In addition, MM-MSC facilitate MM growth in vivo confirming their central role in tumor progression.

E1216
THE NOTCH PATHWAY IN THE INTERPLAY BETWEEN MYELOMA CELLS AND ENDOTHELIUM IN THE BONE MARROW NICH
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Background: Angiogenesis is a hallmark of tumors, and it is a peculiar characteristic in bone marrow (BM) of multiple myeloma (MM) patients. MM is a still incurable disease that strongly depends on interactions with BM microenvironment. Endothelium of MM patients displays malignant behavior as compared to a healthy counterpart. MM displays a dysregulation of the Notch pathway due to Jagged ligands and Notch receptors overexpression. This condition brings to the generation of homotypic and heterotypic interaction loops that sustain MM cells. Moreover, Notch is a developmentally important pathway for BM resident cells, including osteoclast and BM stromal cells (BMSCs), although its role in the crosstalk of MM and endothelium is still to be clarified.

Aims: The aim of this study is to investigate Notch role in MM crosstalk with endothelium exploiting 2D assays and 3D organoid systems to mimic tumor and BM environment (TME).

Methods: The Notch ligands, Jagged1 and 2, were silenced in the MM cell line RPMI8226 (RPMI8226shJAG1/2) using an inducible lentiviral vector carrying two short hairpin RNAs targeting Jagged1 and 2. To mimic the endothelial compartment, both arterial and venous endothelial cells (HAECs) were used and for the stromal compartment, the GFP+HSS cell line. Matrigel and surgical healing assays were set up to investigate Notch role in modulating the angiogenic potential of MM cells co-cultured with HAECs and HPAECs. Angiogenesis in response to MM-derived soluble factors. To develop a TME-like system, a decellularized extracellular matrix (dECM) was used as a physiologic scaffold for organoid generation. dECM was produced by treating murine fibroblast NIH3T3 with ascorbic acid and loaded with cells for organoids generation. We evaluated apoptosis of MM cells in single culture and co-culture with BMSCs or HPAECs by flow cytometry.

Results: Matrigel assay of HPAEC co-cultured with MM cells showed that direct contact increased angiogenic potential of HPAEC to form a grid of tubes; this effect is significantly reduced when HPAECs are co-cultured with RPMI8226shJAG1/2 cells, indicating a key role of Notch signaling in endothelial stimulation. Wound healing assay demonstrated that Notch signaling affects MM cell migration, since it is reduced when Jagged1 is downregulated. Concerning the 3D-organoid generation, our results indicate that the decellularized dECM was a suitable scaffold. Moreover, apoptosis assay revealed that in MM cells displayed an increased survival when cultured in the presence of BMSCs, that consistently with their recognized protective role; no significant difference in MM cell apoptosis was observed in the presence of endothelial cells. On the contrary, we have observed that endothelial cells were protected by MM cells suggesting that MM cells improve angiogenesis by preventing endothelial cells apoptosis.

Summary/Conclusions: These results indicate a novel role for Notch pathway in MM-EC crosstalk suggesting that the Notch pathway activation in MM cells can increase their proangiogenic potential. 3D-organoid mimics BM microenvironment and may be used as a novel tool to recapitulate the interactions of BM and tumor cells beyond the animal models.

References

E1217
MIR-101-3P REGULATES BONE MARROW STROMA-INDUCED DRUG RESISTANCE IN MULTIPLE MYELOMA CELLS BY TARGETING SURVIVIN AND MODULATING CELL-CELL ADHESION
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Background: In multiple myeloma (MM), bone marrow stromal cells (BMSCs) protect MM cells against cell death by direct or indirect interaction. This phenomenon, partly explain drug resistance in MM. Findings of relevant studies indicate activation of some oncogenic or survival pathways including PI3K/mTOR, Ras/MAF, NFκB and Wnt. However, the potential regulatory mechanisms and druggable targets have not been clearly elucidated.

E1218
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Aims: To understand the role of stromal induced drug resistance and to identify new therapeutic targets in myeloma.

Methods: GFP-tagged human myeloma cell lines, 8226, U266 and MM.1s, were co-cultured with MM patient-derived BMMSCs or HS.5 cells with or without BTZ for 24 h. MM cell monolayers were used as controls. Co-cultures were then applied to magnetic cell separation to isolate MM cells for down-stream analyses including western blotting and mRNA or miRNA qPCR arrays. Furthermore, percent apoptosis of gated GFP+ cells was determined using FACS. In other experiments, MM cells were exposed to BMMSCs pre-treated with Brefeldin-A (BFA) or separated with a transwell (TW) insert. For functional analysis, miR-101-3p was overexpressed using lentiviral transduction and survival in murine BMMSCs (BMMSCs) were then seeded on BMMSCs in presence or absence of BTZ. GFP fluorescence-based adhesion, cytotoxicity and annexin-V/PI apoptosis were applied.

Results: qPCR arrays showed that BMMSCs up- or down-regulated several mRNAs and miRNAs in MM cells. Survivin (BIRC5) was confirmed to be conserved in multiple MM cell lines and protein and mRNA levels. In contrast, miR-101-3p was confirmed to be significantly downregulated by stroma in MM cells. Moreover, suppression of miR-101-3p or upregulation of survivin was reversed partially when BMMSCs were pre-treated with BFA but highly significantly when they were separated from MM cells with a TW insert. The same phenomena were observed in invasion FACS analysis indicating that direct cell-cell adhesion was more effective in BMMSC-induced modulations in MM cells. Next we identified that survivin was a direct target of miR-101-3p, overexpression of miR-101-3p suppressed survivin mRNA/protein. As indicator of involvement in stroma-mediated drug resistance, survivin and miRNA-101-3p transfection of the bone marrow stromal cells-BMMSCs showed no significant differences compared to the control cultures. Furthermore, miR-101-3p overexpression or silencing of survivin increased BTZ-induced apoptosis in MM cells in the absence or presence of BMMSCs significantly overcoming stroma-mediated drug resistance. To test whether miR-101-3p could also regulate adhesion of MM cells to BMMSCs, we have demonstrated a significantly reduced adhesion of MM cells to HS.5 and primary MM BMMSCs compared to scrambled control. This finding suggests that miRNA-101-3p regulates cell adhesion-mediated drug resistance (CAMDR) by modulation of BM-BMSC adhesion.

Summary/Conclusions: Our results identify a mechanism whereby BMMSCs induce drug resistance in MM cells by upregulating survivin and downregulating miRNA-101-3p which directly targets survivin. Overexpression of miRNA-101-3p or silencing of survivin sensitizes MM cells to BTZ significantly overcoming stroma-induced drug resistance. These findings disclose a role of survivin-miRN-101-3p axis in regulation of BMMSCs-induced BTZ resistance in MM cells, thus provide a rationale to further investigate the anti-myeloma activity of miR-101-3p in combination with BTZ as a potential therapeutic strategy in MM.

E1218

ARQ-197, A SMALL-MOLECULE INHIBITOR OF C-MET, REDUCES TUMOUR BURDEN AND PREVENTS TUMOUR-ASSOCIATED BONE DISEASE IN A MURINE MODEL OF MYELOMA

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Background: The receptor tyrosine kinase c-Met, its ligand HGF, and their signaling pathways are involved in the pathogenesis of myeloma. In myeloma patients with elevated levels of HGF their prognosis is known to be poor. Therefore, targeting these molecules or their pathway in such patients could be promising therapeutic in myeloma patients who express high levels of HGF, leading to both a reduction in tumour burden and an inhibition of myeloma-induced bone disease.

Summary/Conclusions: In summary, these results suggest that ARQ-197 could be a promising therapeutic in myeloma patients who express high levels of HGF, leading to both a reduction in tumour burden and an inhibition of myeloma-induced bone disease.

E1219

Abstract withdrawn.

E1220

THE GENETIC LANDSCAPE OF THE MURINE 5T MODELS FOR MULTIPLE MYELOMA

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Aims: To unravel the role of the bone marrow mesenchymal stem/stromal cells (BMMSCs) in myeloma cell growth, progression and drug resistance.

Methods: Hypothesizing that the interaction between MM cells and the BMMSCs is bidirectional, we have compared BMMSCs from healthy individuals, mgUS, and MM patients and used our “humanized” bone marrow-like model to characterize the molecular impact of MM cells on BMMSCs. Finally, we have validated targets by generating HS-5 knock-out lines using CRISPR/Cas9 targeting.

Results: Analyzing the BMMSCs of healthy individuals, mgUS, and MM patients and used our “humanized” bone marrow-like model to characterize the molecular impact of MM cells on BMMSCs. The mutational landscape of MM patients has led to the discovery of several potential driver mutations and copy number alterations reflecting this genetic heterogeneity. The mutational landscape of 5T33vv and 5TGM1 models thus represent the most copy number alterations. Over the entire genome, 11% and 17% showed copy number alterations. In summary, these results suggest that ARQ-197 could be a promising therapeutic in myeloma patients who express high levels of HGF, leading to both a reduction in tumour burden and an inhibition of myeloma-induced bone disease.
CD38 monoclonal antibody, Daratumumab (DARA), induces and mediates MM
expression of CD38 and ectoenzymes of the adenosinergic could benefit from treatment with the triple combination PIM447 + pomalidomide.

**Background:**

Triple combination PIM447 + pomalidomide + dexamethasone remarkably reduced the levels of the glucose metabolism-associated enzyme hexokinase II and also reduced glucose uptake by cells. Finally, the efficacy of this combination was confirmed in a plasmacytoma model in CB17-SCID mice, where luciferase gene-marked MM cells were employed in a range of MM translocations; JNJ3 (14;16), U266 (11;14), KMS-18 (4;14), OPM-2 (4;14). TRIM33 knockdown was performed using shRNA plko2 lentiviral plasmids. CellTiter-Glo® was used to determine cell viability following knockdown. Analysis of the TRIM33 expression did not alter cell viability in the t(4;14) cell lines. However, cell viability was found to be increased in JNJ3 (p=0.001) and U266 (p=0.015). Knockdown of TRIM33 expression also not alter cell viability in the non t(4;14) cell lines. However, cell viability was found to be increased in non t(4;14) cell lines (p=0.004) and hyperdiploid cluster (p<0.05). Low TRIM33 expression has also been associated with poor overall survival (GSE26568; p=0.0034). Forty-seven patients were compared with TRIM33 expression in non t(4;14) MM. LMU was used to analyse published datasets to look at TRIM33 expression and correlation with survival in subsets of newly diagnosed MM; GSE19784 (N=320) and GSE26568 (N=551). qPCR was used to validate the changes in expression of the TRIM33 gene signature.

**Aims:**

The aim of this study was to examine TRIM33 expression and to investigate its role as a potential tumor suppressor in MM.

**Methods:**

To analyse TRIM33 expression at basal level and following knockdown in four MM cell lines representing a range of MM translocations; JNJ3 (14;16), U266 (11;14), KMS-18 (4;14), OPM-2 (4;14). TRIM33 knockdown was performed using shRNA plko2 lentiviral plasmids. CellTiter-Glo® was used to determine cell viability following knockdown. Analysis of the TRIM33 expression did not alter cell viability in the t(4;14) cell lines. However, cell viability was found to be increased in JNJ3 (p=0.001) and U266 (p=0.015). Knockdown of TRIM33 expression also not alter cell viability in the non t(4;14) cell lines. However, cell viability was found to be increased in non t(4;14) cell lines (p=0.004) and hyperdiploid cluster (p<0.05). Low TRIM33 expression has also been associated with poor overall survival (GSE26568; p=0.0034). Forty-seven patients were compared with TRIM33 expression in non t(4;14) MM. LMU was used to analyse published datasets to look at TRIM33 expression and correlation with survival in subsets of newly diagnosed MM; GSE19784 (N=320) and GSE26568 (N=551). qPCR was used to validate the changes in expression of the TRIM33 gene signature.

**Results:**

Compared to normal bone marrow, lower expression of TRIM33 was observed at both gene and protein level (p=0.03) in the t(4;14) cell lines, KMS-18 and OPM-2. Conversely, expression was found to be high in the non t(4;14) cell lines, JNJ3 (p=0.001) and U266 (p=0.015). Knockdown of TRIM33 expression also not alter cell viability in the t(4;14) cell lines. However, cell viability was found to be increased in JNJ3 (p=0.004) and U266 (p<0.05). Analysis of a publicly available dataset, GSE19784, showed lower levels of TRIM33 present in patients with a t(4;14) compared to other MM subtypes, particularly (6;14) (p=0.004) and hyperdiploid cluster (p<0.05). Low TRIM33 expression has also been associated with poor overall survival (GSE26568; p=0.0034). Forty-seven seven patients were compared with TRIM33 expression in non t(4;14) MM. LMU was used to analyse published datasets to look at TRIM33 expression and correlation with survival in subsets of newly diagnosed MM; GSE19784 (N=320) and GSE26568 (N=551). qPCR was used to validate the changes in expression of the TRIM33 gene signature.

**Summary/Conclusions:**

Our preclinical data suggest that myeloma patients could benefit from treatment with the triple combination PIM447 + pomalidomide + dexamethasone and would support future clinical trials with this combination.

**E1223**

**THE PAN-PIM KINASE INHIBITOR, PIM447, POTENTLY SYNERGIZES WITH POMALIDOMIDE PLUS DEXAMETHASONE IN PRECLINICAL IN VITRO AND IN VIVO MODELS OF MULTIPLE MYELOMA**

**Aims:**

The combination index (CI) was calculated with Calcusyn software based on results from MTT assay. Effects on apoptosis and cell cycle were evaluated by flow cytometry. Glucose uptake was analyzed by incubation with 2-NBDG. The mechanism of action was explored by analysis of different protein levels by western blot. Finally, a plasmacytoma model in CB17-SCID mice was employed. The combination index (CI) was calculated with Calcusyn software based on results from MTT assay. Effects on apoptosis and cell cycle were evaluated by flow cytometry. Glucose uptake was analyzed by incubation with 2-NBDG. The mechanism of action was explored by analysis of different protein levels by western blot. Finally, a plasmacytoma model in CB17-SCID mice was employed.

**Methods:**

**Results:**

**Summary/Conclusions:**

Taken together these data suggest that although IL-6 is one of the most deregulated genes in MM-derived BMMSCs, it certainly is not the sole contributor to BMMSC-induced MM cell growth and drug resistance.
enhancer of the TRIM33 signature that potently decreased the viability of the
OPM.2 cell line. This study suggests that enhancing the TRIM33 gene signature
could potentiate the tumor suppressive effect of TRIM33 and identify novel
therapies for this subset of MM.

E1225
LONG NON-CODING RNAs EXPRESSION HETEROGENEITY AND
FUNCTIONAL INVOLVEMENT IN MULTIPLE MYELOMA

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Background: Increasing amount of evidence indicates that deregulation of
long non-coding RNAs (lncRNAs) is a common feature of cancer and therefore,
itself may uncover new molecular oncogenic mechanisms. In multi-
tple myeloma (MM), altered expression of small number of lncRNAs has been
associated with decreased disease-free and overall survival, suggesting that
these elements may play a more important role in MM than previously antici-
plated. Nevertheless, an extensive high-throughput analysis that characterizes
the deregulation of lncRNAs in MM has not yet been performed.

Aims: We aim to characterize the IncRNA transcriptome of MM and its hetero-
genility, and determine whether altered IncRNAs have a functional involvement
in this disease.

Methods: Paired-end strand-specific RNA sequencing (ssRNA-seq) was performed
in 38 purified plasma cell (PC) samples from MM patients, as well as in 5 tonsil
PCs (TPCs) and in 3 bone marrow PCs (BMPCs) of healthy donors as
controls. We also performed ssRNA-seq of populations from B cell differen-
tiation (Naïve, Germinal Center, Memory and PC) to study the heterogeneity
of lncRNAs expression we performed sample level enrichment analysis (SLEA),
in which each individual IncRNA was compared to BMPCs. To determine the
epigenetic regulation of IncRNAs we used whole-genome bisulfitel sequencing and
CHIP-seq, shRNA-mediated knockdown using 2 different shRNAs and
MT-1 microRNA inhibition (MT-1 knockdown) and reprogramming of V (cell
death) assays were utilized to study the functional effect of IncRNA overexpression.

Results: We identified 40.552 novel IncRNAs in MM samples that were present
in at least 3 of the 38 patients. Principal component analysis demonstrated that
TPCs and BMPCs cluster separately, suggesting that, in spite of being
the same tissue type, their transcriptomes are very different. We observed that
the expression of IncRNAs was more heterogeneous than that of coding genes.
More importantly, SLEA showed 11.067 lncRNAs that were overexpressed and
5.601 underexpressed in >40% of patients. Thus, the number of deregulated
genes analyzed by SLEA was much larger than the 70 lncRNAs that appeared
5.601 underexpressed in >40% of patients. Thus, the number of deregulated
lncRNAs was more heterogeneous than that of coding genes. Importantly,
SLEA showed 11.067 IncRNAs that were overexpressed and
5.601 underexpressed in >40% of patients. Thus, the number of deregulated
genes analyzed by SLEA was much larger than the 70 lncRNAs that appeared
dereregulated in MM and not at different stages of B-cell differentiation. DNA methylation analysis demon-
strated that CpGs located upstream of LINC-SMILO showed a significant
hypomethylation in MM, that was even more pronounced in MM samples.
We also have observed a gain of active chromatin modifications in the promoter
region of LINC-SMILO in MM patient samples. These data suggest that
epige-
netic elements, namely chromatin, in the gain of active histone modifications,
may be the cause of LINC-SMILO overexpression in MM. Knock-
down of LINC-SMILO in 3 different cell lines (MM.1S, MM.1R and KMS-11)
resulted in reduced proliferation and induction of apoptosis, indicating this IncR-
NA is essential for the survival of MM cells.

Summary/Conclusions: All together, these data demonstrate that alteration of
IncRNAs is an important and unexplored feature of MM. Moreover, over-
expression of LINC-SMILO is required for the survival of MM cells and could rep-
resent a potential therapeutic target for the treatment of this disease.

E1226
ROLE OF EPHA3 IN MULTIPLE MYELOMA: A PERSPECTIVE FOR A
NOVEL TARGET THERAPY?

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Background: The tyrosine kinase Eph receptor A3 (EphA3) has recently
emerged as a potential therapeutic target, since it has been overexpressed in
many cancers, including some hematological malignancies (Keane et al.
2012). Furthermore, EphA3 has been found overexpressed not only in neo-
plastic cells, but also in the microenvironment of different human cancers,
where its targeting inhibits tumor growth by disrupting supportive stroma and
vasculature (Val et al. 2014).

Aims: Due to the absence of relevant information about the role of EphA3 in mul-
tiple myeloma (MM), we aimed to evaluate the expression of this molecule in pri-
mary bone marrow plasma cells (BMPCs) from MM patients and MM cell lines
compared to healthy controls (HCs). In addition, using a “loss of function” approach
by mRNA silencing and an anti-EphA3 monoclonal antibody (EphA3mAb), we
studied in vitro plasma cells (PCs) viability and movement. Finally, we analysed
the in vivo effects of EphA3mAb in a MM mouse xenograft model.

Methods: EphA3 mRNA and protein where investigated in 15 MM BMPCs, 11
MM cell lines and 10 HCs by qRT-PCR and flow cytometry. The effects of EphA3 targeting by lentiviral RNA silencing (shRNA) and anti-EphA3mAb on
PC trafficking and viability were studied by adhesion assay on fibronectin and
on bone marrow stromal cells (BMSCs), invasion assays and proliferation MTS
 assay, respectively. Gene expression profiling (GEP) was performed in shEph-
A3 versus shControl cells. Furthermore, the effects of EphA3mAb were analyzed in a MM xenograft model by measuring tumor size and by assessing
angiogenesis, proliferation and apoptosis rate on tumor biopsies using immuno-
histochemistry (anti-CD31, anti-ki67 and TUNEL assay, respectively). Statistical
significance was determined by the t-test or One-way ANOVA analysis.

Results: EphA3 was found overexpressed in primary MM BMPCs and MM
cell lines when compared with HCs (figure 1A-B). The EphA3 loss of function
by siRNA and by EphA3mAb significantly inhibited in vitro the ability of MM
PCs to adhere to fibronectin, to BMSCs and to invade (figure 1C-E), without
affecting cell proliferation and viability (data not shown). GEP showed that
knockdown of EphA3 modulated some molecules that regulate adhesion,
migration and invasion processes. Importantly, the treatment with EphA3mAb
in vivo significantly reduced tumor size and inhibited angiogenesis, as revealed
by decrease of CD31+ vessels at immunohistochemistry (data not shown).

Figure 1.
Summary/Conclusions: Our findings suggest that EphA3 is a novel regulator of MM PC trafficking, in part via effects on adhesion and invasion; its targeting using EphA3mAb inhibits tumor growth, possibly by reducing angiogenesis, though other possible mechanisms of tumor death cannot be excluded. These data, together with the favourable clinical properties of a humanized EphA3mAb reported in a phase I trial on acute myeloid leukemia and myelodysplastic syndrome (Swords et al. 2016), support EphA3 targeting as a new potential therapeutic opportunity for MM that would warrant to be further investigated.

E1227

PROGNOSTIC SIGNIFICANCE OF AMP1Q21 IN MULTIPLE MYELOMA
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Background: Multiple Myeloma (MM) is a genetically heterogeneous and complex disease with widely diverging survival times from months to years. Amplification of locus 1q21 (amp1q21) is among the most commonly reported genetic abnormalities in MM, but its prognostic value remains unclarified.

Aims: To define the frequency of amp1q21 in MM and its correlation with other chromosomal abnormalities, clinical course and prognosis.

Methods: In 134 patients (pts) with newly diagnosed MM from December, 2009 to March, 2016, 67 male and 67 female, median age 70 years (30-81), we performed FISH with locus-specific and centromere DNA probes (XL 1p32/1q21, XL IGH plus, XL t(11;14), XL t(4;14), XL t(14;16), XL t(14;20), XL t(6;14), XL cMYC BA, XL 5p15/9q22/15q22, XL P53 [MetaSystems], D13S25 [Cyto Cell]). Induction therapy with bortezomib-based courses was initiated for 131 pts, 3 pts with smoldering MM remained under observation. Response was evaluated according to the IMWG criteria for 127 pts, because 4 pts died in induction. 48 pts were underwent ASCT. The median follow-up of group was 19.3 months (3.2 – 77.4). Progression was diagnosed in 69 pts, in 12 of them FISH-analysis was performed also in disease progression.

Results: Chromosomal aberrations were revealed in 133 of 134 (99%) pts. T1q14/Tq22 was detected in 42.5% (57/134), hypodiploidy in 57.5% (77/134), hypodiploidy in 2.4% (3/134) pts. In 11.2% (15/134) a concurrent t1q14/Tq22 and a trisomy were found. The IGH translocations t(11;14), t(4;14), t(14;16), t(14;20), t(6;14) were observed at a frequency of 16.4%, 12.7%, 3.2%, 2.2%, 0.7% respectively, chromosomal partner is not found in 6.7%. Del(13q) was detected in 40.3% (54/134), del(17p) in 17.3% (23/134), del(13q) and 3 copies of 1q21 in 60.4% (31/51) cases. Cases with Amp1q21 had a high incidence of del(13q) (OR=2.71 (1.32-5.55); p=0.006) and t(14;14) (OR=4.49 (1.47-13.51); p=0.005), as well as higher LDH levels (OR=2.27 (1.09-4.72); p=0.027). From 12 pts investigated in progression amp1q21 was found in 9 pts (75%); in 2 cases amp1q21 was not found at diagnosis and was revealed in disease progression only; in 7 cases - amp1q21 was detected at diagnosis and in progression, and its copy number did not change. The difference in response after induction between pts with or without amp1q21 was not statistically significant: CR – 11.8% versus 14.5%; VGPR – 39.2% versus 27.6%; PR – 37.2% versus 27.6%; therapy resistant 11.8% versus 30.3% (p=0.07). Pts with amp1q21 had significantly worse 5-year overall survival (OS) (43.5% vs 79.4%; p=0.07). According to copy number of 1q21 the 5-year OS pts carrying 3 or >3 copies of 1q21 were 67.3% and 20.9% (p=0.0016) (Figure 1). On multivariate analysis 3 copies of amp1q21 (HR=4.29, p=0.0094), tMYC/8q24 (HR=6.51, p=0.0082), del(17p) (HR=3.46, p=0.007) were found to be an independent adverse predictors of shorter OS.

Amp1q21 can appear in the course of MM, therefore FISH-analysis of locus 1q21 should be performed at diagnosis, as well as in disease progression.

E1228

ADAPTIVE IMMUNE RESPONSE IN PLASMA CELL DYSCRASIAS: IMMUNE PROFILING AND DETERMINATION OF CIRCULATING B CELL LEVELS AS A SURROGATE ASSAY FOR BONE MARROW TESTING
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Background: Immune paresis is commonly identified in patients with plasma cell dyscrasias (PCD). Often, in newly presenting multiple myeloma (MM), it is associated with intractable infections for which the patient first seeks medical help. Furthermore, recent evidence suggests the importance of assessing levels of bone marrow (BM) derived B cells for risk stratification of the MM patients as reduced levels of B-cells in the BM have been associated with poorer outcomes and reduced progression free survival1. This cellular measure of adaptive immune function (ie: B cell enumeration) is, however, seldom analysed in the peripheral blood (PB) of patients with PCD.

Aims: This study was designed to examine measures of the adaptive immune response in PCD patients, by measuring relative and absolute numbers of T, B cell subset, NK and NKT cells at different stages of PCD, and to determine if the PB-B cell component can act as a surrogate marker for B cell enumeration in MM.

Methods: PB and BM lymphocyte subset analysis was performed on samples obtained from a range of PCD patients (n=70) using directly conjugated monoclonal antibodies (MAB) and multicolour flow cytometry, carried out on a FACSAria III cell sorter (BD, Oxford, UK). Serum protein electrophoresis was performed to identify and quantify paraproteins, and uninvolved Ig levels were quantified by nephelometric methods. sFLC were also measured using the Freelite assay on the SPAPlus instrument (Binding Site, Birmingham, UK).

Results: Data is presented on 102 PB samples obtained from 70 PCD patients at different stages of disease, including monoclonal gamopathy of undetermined significance (MGUS), smoldering myeloma (SMM), and MM at diagnosis (MMD), throughout treatment (MMT) and at relapse (MMR). Quantification of circulating lymphocyte subsets showed reduced, absolute, numbers of B cells (56/102), T cells (19/102), T eff cells (32/102), CTLs (17/102), NK cells (32/102) and NKT cells (72/102). Furthermore, these reduced B cell levels were more frequently seen in the MMD and MMT groups (50% of samples) compared with the other PCD groups (10-25% of samples). Lymphocyte subset analysis was also performed on paired PB and BM samples from 14 patients with MM and a significant, positive, correlation was seen between relative numbers of B cells in both PB and BM (r<0.0001, r=0.94). No clearcut correlations were found between reductions in uninvolved sFLC levels, and numbers of cells involved in the adaptive immune response.

Summary/Conclusions: The results presented here are further evidence of immune paresis in PCD with specific effects seen at the cellular level. The highest frequency of reduction was in B lymphocytes and NKT cells, in keeping with reduced levels of circulating BM B cells, followed by T cells, particularly T eff cells which have a crucial role in B cell Ig production. Relative B cell levels in BM were significantly correleated with B cell levels in PB and we suggest that monitoring of B cell levels in the PB of PCD patients may serve as a surrogate assay for enumeration of B cells in BM.

References

E1229

NOVEL MONOCLONAL ANTIBODY THERAPY TARGETING CD26 IN MULTIPLE MYELOMA
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Background: Bone disease is a hallmark of multiple myeloma (MM) and targeting osteoclasts (OCs) to alleviate bone destruction is a component of the standard care for MM. CD26 is a 110-kDa cell surface glycoprotein with DPP IV enzyme activity and has well-defined roles in T cell activation and several tumor developments, including malignant lymphoma. However, little is known about the role of CD26 in regulating bone remodeling.

Aims: In this study, we examine the CD26 expression in human normal OCs and OCs of MM patients. We explore the function of CD26 in osteoclastogenesis and investigate the effects of humanized anti-CD26 monoclonal antibody (CD26mAb) on human OCG. We further define the molecular targets of CD26 signaling cascade in OCG and explore the therapeutic potential of CD26mAb for treating MM.

Figure 1.

Summary/Conclusions: Our results show that amp1q21 has a significant impact on OS MM pts in cases of more than 3 copies of locus only. In cases of 3 copies of 1q21 OS pts is comparable with OS in group without amp1q21.

haematologica | 2017; 102(s2) | 503

Madrid, Spain, June 22 – 25, 2017
Methods: Human BM-MMCs derived from normal human subjects or MM patients were cultured with M-CSF plus sRANKL with or without G2626Am for OC formation for TRAP staining and functional assay. To assess the mechanisms of action of CD26Am on OCs, RANK signaling proteins were examined by immunoblotting.

Results: CD26 is expressed on normal human OCs and is intensely expressed on activated OMs in MM. The low AhR with sRANKL induced human OC differentiation, in association with CD26 expression on monocyte-macrophage lineage cells. CD26 expression was accompanied by increased phosphorylation of MKK3/6 and p38MAPK, which is crucial for human OC differentiation with its downstream activation of microphthalmia-associated transcription factor (miT)/CEBPβ, an important regulator of MM. CD26, measured in the number of multinucleated OCs (>3 nuclei) by TRAP/CD26 staining and down-regulated the secretion of TRAP-5b and type 1 collagen. It decreased the size of OCs and the number of nuclei per OC, with significantly defective bone resorption activity. It was revealed that in the presence of CD26Am, which induced OC precursor cells (MKK3/6/miT) phosphorylation pathway was specifically, rapidly inactivated and subsequently, its downstream miT/miF-phosphorylation was persistently inhibited. Thus, OC maturation with its bone resorption was impaired by suppressing the expression of TRAP and OC fusion proteins. In contrast, MKK3/6-p38MAPK-miF was not phosphorylated at all in immature OCs after RANKL stimulation, regardless of the absence or presence of CD26Am. These results suggest that CD26Am blocked RANKL induced p38MAPK phosphorylation in OC precursor cells, but not in OMs. The activation of other MAPKs including ERK and SAPK/JNK, or NFkB was rapidly induced in response to RANKL both in OC precursor cells and MM cells, and the absence of phosphorylation of CD26Am did not directly affect mature OC functions. Next, although CD26Am did not demonstrate direct inhibition of proliferation of MM cells, to further investigate the role of CD26 in MM cells in the BM, co-cultures of 10 MM cells lines with 11 MM cell lines with CD26-stained OCs were performed. We examined the expression of CD26 in MM cells. Although CD26 expression was only slightly detected in any of MM cell lines in mono-culture, CD26 expression level was upregulated in all MM cell lines, co-cultured with OCs by flow cytometry and immunohistochemistry. CD26 protein level in these cell lines was also increased by immunoblotting or ELISA. To further explore the CD26 expression in the BM of MM patients, we performed immunohistochemical staining on decalcified biopsy specimens. CD26/CD138 positive plasma cells were detected around CD26 positive OCs and certain endothelial vascular cells in several cases. Anti-miycoplasma efficacy of CD26Am on MM cells, co-cultured with OCs was also confirmed.

Summary/Conclusions: Our data imply that the blockade of CD26 signaling with CD26Am impairs the development of human functional OCs. Targeting CD26 in both OCs and MM cells with CD26Am may be a promising novel therapeutic strategy in MM-associated bone disease and MM progression.

E1231

THE ANTI-MYELOMA ACTIVITY OF PERK KINASE INHIBITOR IN TARGETING MORE THAN 50 UPR-RELATED GENES INVOLVED IN THE PROLIFERATION OF MM CELLS

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Background: Due to the immunoglobulin production, multiple myeloma (MM) plasma cells are dependent on the unfolded protein response process (UPR), which controls protein production and ensures its proper translation and folding. A study by Michallet et al (2011) showed that knockdown of one of the three well-known arms of the UPR, PERK (protein kinase R (PKR)-like kinase) in MM cells resulted in autophagic cell death. This outcome indicated the importance of PERK activation for the proliferation of plasma cell to myeloma cell but also its ability to impede the apoptotic effect. In this study we used a small ATP competitive molecular inhibitor, GSK2606414, that acts by targeting PERK enzyme activity in its inactive DFG conformation at the ATP-binding pocket. We demonstrated that while displaying ≥385 fold selectivity over c-Kit, aurora B, BRK and many other kinases.

Aims: In this study we aimed to use a small ATP competitive molecular inhibitor, GSK2606414, that acts by targeting PERK enzyme activity in its inactive DFG conformation at the ATP-binding region, while displaying ≥385 fold selectivity over c-Kit, aurora B, BRK and many other kinases.

Methods: We initially screened 25 CD138+ MM patients and 6 human myeloma cell lines (HMCls) for PERK mRNA expression. Our results showed that PERK mRNA is highly expressed in almost all patients (5-10 fold higher than the mean PERK expression of HMCls).

Results: We test the effect of GSK2606414 on the proliferation of MM cells. 4 HMCls were treated with different doses of GSK2606414 at two time points (24 and 48 hours). Treatment of cells with 3-30μM GSK2606414 resulted in a dose-dependent inhibition of cell proliferation in all HMCls ranging for 20-95% reduction of proliferative activity, thus, indicating the dependency of these cells on PERK activation. Treatment of the other cells (H929 and L363) with 20μM GSK2606414 for 24 hours resulted 25% and 15% increase in apoptotic cells by Annexin-Pi staining respectively compared to the untreated cells. However, the most important finding was a significant synergistic effect of GSK2606414 with bortezomib in these cells. Specifically when H929 and L363 cells were treated with 5nM bortezomib in combination with 20μM GSK2606414, synergistic effect was seen where apoptotic cells reached 99% and 77% respectively, compared to bortezomib-treated cells (87% and 42% respectively). Furthermore, in experiments with 3-30μM GSK2606414, the most important finding was a significant synergistic effect of GSK2606414 on the proliferation of MM cells. 4 HMCls were treated with different doses of GSK2606414 at two time points (24 and 48 hours). Treatment of cells with 3-30μM GSK2606414 resulted in a dose-dependent inhibition of cell proliferation in all HMCls ranging for 20-95% reduction of proliferative activity, thus, indicating the dependency of these cells on PERK activation. Treatment of the other cells (H929 and L363) with 20μM GSK2606414 for 24 hours resulted 25% and 15% increase in apoptotic cells by Annexin-Pi staining respectively compared to the untreated cells. However, the most important finding was a significant synergistic effect of GSK2606414 with bortezomib in these cells. Specifically when H929 and L363 cells were treated with 5nM bortezomib in combination with 20μM GSK2606414, synergistic effect was seen where apoptotic cells reached 99% and 77% respectively, compared to bortezomib-treated cells (87% and 42% respectively). In addition, the effect of GSK2606414 in combination with bortezomib in the proliferation of H929 and L363 cells was examined. As seen in the apoptosis assay, pretreatment of H929 and L363 cells with 5nM bortezomib resulted in 40% and 30% reduced cell proliferation in H929 and L363 respectively compared to bortezomib only treated cells. Under ER stress conditions, the activation of ATF6 and PERK/eIF2α leads to the induction of ATF4 translation and results in the upregulation of CHOP. To determine the gene target effects of GSK2606414, ATF4 and CHOP mRNA expression levels were determined in H929 cell line after 24 hour of treatment. Treatment with GSK2606414 alone did not alter the expression levels of CHOP but reduced more than 50% the expression levels of ATF4. When combined with bortezomib CHOP and ATF4 levels were reduced 28% and 50% respectively while treatment with bortezomib and GSK2606414 showed a significant reduction in the levels of CHOP and ATF4 by 50-100%. Changes in RNA expression of 84 UPR-related genes were analyzed in H929 cells. Specifically H929 cells were pre-treated with GSK2606414 and then subjected to ER stress conditions by treatment with tunicamycin. (TM). After 24 hours of treatment, 50 genes were found to be transcriptionally regulated by 5-fold in response to TM. (CCEBPB, CEBPB, ESS1, IFIT1, ITAC, KLF4, LPL, NALP3, NQO1, PPP1R15A, etc.) were downregulated by >5 fold, whereas 10 of these genes (HERPUD1, EIF2AK3, CREBL3, HSPA2, HSPA1B, etc.) were upregulated similarly.
Summary/Conclusions: In conclusion, given the on-target pharmacological effects of PERK inhibitor on MM, development of PERK inhibitors may offer a therapeutic advantage that would affect MM pathogenesis and treatment.

E1232 ENVIRONMENTAL CONTROL OF PLASMA CELL FITNESS IN MULTIPLE MYELOMA: MALIGNANT CO-OPTATION OF ARGININE AS NOVEL IMMUNE CHECKPOINT

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Background: The bone marrow (BM) environment plays a crucial role in the incurable plasma cell (PC) malignancy multiple myeloma (MM). Our previous works showed that arginine (Arg) is a key nutrient for MM (Carcinogenesis, 2012; 33: 2815-2823). We aim to investigate whether the environmental arginine availability could modulate the PC fitness within the bone marrow niche.

Methods: We have studied the medical records of 479 patients with MM (M/F: 313/166, median age: 67 years, range 28-88). The presence of environmental Arg deficiency in our patients was assessed on the basis of median plasma Arg levels (289 µmol/l vs 309 µmol/l, p<0.001). Patients with Arg deficiency had higher Arginine:Glu concentration ratio (1.7 vs 1.3, p<0.001). We analyzed patient's characteristics between the EM group and the rest of the patients, performed with χ², one-way ANOVA and Mann Whitney U test. Prognostic factors for EM and overall survival (OS) were studied by using logistic regression and cox regression analysis, respectively; OS was plotted by Kaplan-Meier; p<0.05 was considered as statistically significant.

Results: Patients with EM were more often men with a higher median age; hematocrit, platelets and albumin were lower whereas β2 microglobulin, lactate dehydrogenase (LDH) and calcium were higher in the EM group compared to the rest of MM patients (p<0.05). The percentage of patients with abnormal estimated Glomerular filtration (eGFR) calculated by chronic kidney disease epidemiology collaboration (CKD-EPI) creatinine equation (eGFR<40ml/min/1.73m²) was higher in the EM group compared with the rest of the patients (69% vs 17%, p<0.001). In accordance with the International Staging System (ISS), advanced MM stage (i.e. ISS3) was observed more often in the EM group compared to the rest (65% vs 31%, p<0.001). High risk cytogenetics including t(4;14), t(14;16) and del17p were present in 48% of patients in the EM group vs 21% of patients in the rest of MM patients (p<0.001). The incidence of EM included infections (21%), renal (15%), relapsed/refractory disease: 26%, other causes: 6%. Univariate logistic regression analysis demonstrated that ISS, revised ISS (R-ISS), abnormal LDH, hemoglobin <10g/dl, high risk cytogenetics, and CKD-EPI <40ml/min/1.73m² were independent prognostic factors for EM. In the multivariate analysis ISS and abnormal eGFR were the only independent prognostic factors for EM. When we incorporated ISS and eGFR in a single prognostic model (CKD-EPI/ISS) we identified 3 distinct prognostic groups: 1) low risk group including patients with ISS1 and CKD-EPI ≤40ml/min/1.73m², 2) high risk group including patients with ISS3 and CKD-EPI <40ml/min/1.73m² and 3) intermediate risk group including patients that did not fit in either low or high risk group. The incidence of EM in each group was 8.1% 39% and 15.3%, respectively (OR: 2.8, 95% CI:1.9-4.1, p<0.001). Multivariate cox regression analysis of prognostic factors for OS in the whole population demonstrated that CKD-EPI/ISS model was the strongest independent prognostic factor for OS (HR= 0.38, 95% CI: 0.29-0.49, p<0.001).

Summary/Conclusions: Based on our data, the combination of eGFR estimated by CKD-EPI with ISS (CKD-EPI/ISS) represents a powerful independent prognostic model for EM and OS, in the era of novel agents. The markers constituting ISS and ISS are cheap and available for most of MM patients, therefore the CKD-EPI/ISS prognostic model may have a wide application. Nevertheless, the establishment of CKD-EPI/ISS model requires further validation.

E1233 ACTIVATED AND EXPANDED NATURAL KILLER CELLS FROM MULTIPLE MYELOMA PATIENTS DESTROY TUMOR DRUG RESISTANT CELLS AND CLONOCENIC TUMOR CELLS

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Background: Multiple myeloma (MM) remains an incurable disease. Novel therapeutic strategies targeting drug resistant cells (DRC) and clonogenic tumor cells (CTC) are needed. Our group has conducted a phase I clinical trial with activated and expanded autologous NK cells (NKAES) in patients with refractory MM with a relevant clinical effect. Likewise, it has been possible to discriminate DRCs in MM by side population (SP) detection.

Aims: The aim of this study was to characterize DRC and to check the activity of NKAES against these DRCs and CTCs while preserving the hematopoietic progenitor cell.

Methods: Flow cytometry of the side population was performed by Dye Cycle Violet efflux detection to characterize DRC of MM cell lines and bone marrow samples from MM patients. The side population was purified by sorting and characterized by RNAseq. NK cells from MM patients' peripheral blood were co-cultured and cocultured with the genetically modified K562-mb15-41BBL cells obtained and cocultured with the genetically modified K562-mb15-41BBL cells in order to obtain NKAES. The activity of NKAES cells against SP was evaluated by time lapse microscopy and the activity against CTCs was evaluated by methylcellulose assay. In vitro safety against CD34 + progenitors was evaluated by time-resolved fluorescence cytoxicity with europium-TDA and culturing methylcellulose with CD34+ cells to evaluate viability.

Results: SP cells from both cell lines and samples from different stages of MM showed overexpression of stemness markers. Patient NKAES cells were shown to have much higher adhesion, migration and detection capacity of MM tumor cells than NK cells from the same patient. After SP purification by sorting, NKAES were used to detect activated NK cells in patient samples. Experiments on CD34+ and cocultured with the genetically modified K562-mb15-41BBL cells in order to obtain NKAES. The activity of NKAES cells against SP was evaluated by time-lapse microscopy and the activity against CTCs was evaluated by methylcellulose assay. In vitro safety against CD34 + progenitors was evaluated by time-resolved fluorescence cytoxicity with europium-TDA and culturing methylcellulose with CD34+ cells to evaluate viability.

Summary/Conclusions: Based on our data, the combination of eGFR estimated by CKD-EPI with ISS (CKD-EPI/ISS) represents a powerful independent prognostic model for EM and OS, in the era of novel agents. The markers constituting ISS and ISS are cheap and available for most of MM patients, therefore the CKD-EPI/ISS prognostic model may have a wide application. Nevertheless, the establishment of CKD-EPI/ISS model requires further validation.
UNMASKING THE RETROTRANSPOSON-ORCHESTRATED PRODUCTION OF SOLUBLE RANKL IN MULTIPLE MYELOMA CELLS

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Background: Growing evidence suggest that production of soluble receptor activator of nuclear factor-kappa B ligand (sRANKL) directly by myeloma cells is causally related to the generalized bone loss in multiple myeloma (MM). Notably, sRANKL may be produced either by proteolytic cleavage of membrane-bound RANKL or by alternative splicing of TNFSF11 gene (TNFSF11 variant 2, sRANKL mRNA). Recent analysis argues against proteolytic processing of the membrane-bound form being the main mechanism of sRANKL production by myeloma cells. Accumulative data indicate that sRANKL mRNA presents a restricted transcriptional pattern, namely is expressed predominantly in malignant cell types. Accordingly, sRANKL mRNA (over)expression in primary MM cells and human MM cell lines has been validated in three independent studies. Furthermore it was recently demonstrated that sRANKL mRNA proximal promoter and exon 1 are of retroviral origin, residing within a large genomic cluster of transposable elements (TEs).

Aims: To unmask the TE-shaped transcriptional and epigenetic apparatus impelling the expression of sRANKL mRNA in a cell-type and context-specific manner.

Methods: RepeatMasker software was used to reveal the presence of integrated TEs in the genomic segment comprising TNFSF11, TNFSF11 RNA-seq data, generated by the GTEX project across 51 normal human tissues, were analyzed via GTEX Portal. TNFSF11 RNA-seq data from 4 bone marrow samples and 8 white blood cells samples, generated from the PRJEB4337 and PRJNA182351 BioProjects, were analyzed via the NCBI portal. TNFSF11 transcription factor (TF) ChIP-seq data were downloaded from the UCSC Genome Browser Database. Data on TNFSF11 proximal promoter methylation status in 63 cell lines were downloaded from the HAIB Methyl450 ENCODE track.

Results: RNA-Seq data from 51 normal human tissues show that sRANKL mRNA is expressed exclusively in testis, which is in accordance with the retroviral origin of the transcript. Data analysis from the PRJEB4337 and PRJNA182351 BioProjects further validates the null expression of sRANKL mRNA in normal human bone marrow and white blood cells. Methylation status of sRANKL mRNA promoter in 5 lymphoblastoid cell lines (LCLs) signifies that the retroviral promoter remains heavily methylated in these cell types. TNFSF11 TF ChIP-seq data show that 5 of 161 TFs can bind to the TE-derived sRANKL mRNA promoter region. Four of the five TFs (EBF1, PAX5, IKZF1, and PU.1) bind to this genomic segment exclusively in LCLs, signifying a cell-type specific transcriptional regulation. Notably, all 4 TFs are known to play a major role in normal and/or malignant lymphopoiesis. Furthermore, IKZF1 and PU.1 represent direct targets of immunomodulatory drugs (IMiDs) for down-regulation.

Summary/Conclusions: Transcription of sRANKL mRNA is driven by a retroviral promoter which remains heavily methylated, thereby inactive, in normal lymphocytes. Epigenetic derepression of this promoter during the course of myeloma pathogenesis is critical in the (over)expression of sRANKL mRNA by myeloma cells represents a plausible scenario. Should the IKZF1 and the PU.1 TFs act as enhancers of sRANKL mRNA expression, directly contributing to upregulation of sRANKL production in MM, it is a tantalizing hypothesis that warrants further investigation especially because this type of transcriptional boost could be exploited for tailored treatment with IMiDs. That Lenalidomide treatment downregulates the amount of sRANKL in the serum of patients with MM through inhibiting PU.1 expression (Breitkreutz et al., Leukemia 2008) is in accordance with the above and further raises the interest on the mechanisms promoting the anti-osteoclastogenic properties of IMiDs.

ADENOSINE IN THE MYELOMA BONE MARROW NICHE: IMMUNE MODULATION AND KEY ROLE IN DISEASE PROGRESSION

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Background: The tumor microenvironment is rich in extracellular mono- and di-nucleotides (ATP, NAD) which are metabolized by cell surface ectoenzymes to produce adenosine (Ado), a nucleoside involved in the control of inflammation and immune responses. Multiple myeloma (MM), a plasma cell malignancy that develops within the bone marrow (BM) niche, overexpresses CD38, a molecule with complex functions. As a nucleotide-metabolizing ectoenzyme, CD38 catalyzes the initial disassembly of NAD (to cADPR and ADPR), which is followed by adenosinergic activity, providing that CD38 is operating in the presence of other ectoenzymes (CD203a and CD73).

Aims: To demonstrate that adenosinergic pathways contribute to customize homeostasis in MM.

Methods: Evaluation of the expression of adenosinergic coenzymes was assessed by immunohistochemical and flow cytometric analysis on cell lines, and primary myeloma cells and BM biopsies from patients with MM or with monoclonal gammopathy of undetermined significance (MGUS) in the context of MGUS (MM by definition). BM specimens from patients with myeloma overexpress CD38, and the adenosinergic metabolic strategy to silence immune effectors during the progression, and patients with symptomatic MM usually have higher levels of Ado. This is reflected statistically in the International Staging System (ISS) for MM. Preliminary studies showed that MvS deriving from MM patients are peculiar in that i) they are enriched for CD38, CD203a and CD73 ectoenzymes and ii) are able to generate Ado. These MvS may adhere or fuse with neighboring cells, egress the BM niche and eventually reach the bloodstream. Thus, one aspect of the dynamics of available anti-CD38 antibodies for MM therapy is the production of MVs that can be captured by Fc receptor-competent folate treatment with IMiDs. This context (Pérez-Persona E. et al., 22nd Congress of the European Hematology Association, 2021). Ado production offer the opportunity to develop an adenosinergic biomarker for patients in whom a tailoring of therapies may have an impact on outcome. Further studies will determine the validity of Ado levels as a biomarker in MM and its potential to predict MM progression and treatment response.
TREATMENT OPTIMIZATION FOR MULTIPLE MYELOMA: SCHEDULE-DEPENDENT SYNERGISTIC CYTOTOXICITY OF POMALIDOMIDE AND CARFILZOMIB ON AN IN VITRO AND EX-VIVO MODEL

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Background: In recent years significant progress has been made in the understanding of Multiple Myeloma (MM) biology. These advances have translated into the development of new drugs and a different approach to treatment, which has ultimately translated into an unprecedented rate of complete remissions. Immunomodulatory drugs (IMiDs) and proteasome inhibitors (Pis) form the backbone of modern MM treatment, but new and more targeted treatments are under development and are being tested in the context of clinical trials. Pomalidomide (POM) is a third-generation IMiD with immunomodulatory, antiangiogenic, and direct anti-MM activities, and greater in vivo potency than its sister Lenalidomide. Carfilzomib (CAR) is a second-generation irreversible PI that is structurally and mechanistically distinct from Bortezomib. Preclinical study suggested that the timing and dosing schedules of IMiDs in combination with Pis treatment is critical, proposing a first evidence that established treatment regimens need to be carefully re-evaluated to maximize the anti-tumor effects.

Aims: In this study we tried to optimize the anti-MM therapy using the new class of agents of IMiDs and new generation Pis, by evaluating a possible synergistic effect between POM and CAR.

Methods: For the purpose of this study we used five bona fide MM cell lines (MM1.S, OPM-2, NCI-H929, KMS12.BM and U266), a human bone marrow stromal cell line (HS-5 cells) and primary samples from newly diagnosed MM patients. Apoptosis analysis was done up to 48 h after administration of the first drug. For each drug, three different concentrations were used: low dose, intermediate dose and high dose. Since the BM microenvironment is a complex and active system, with potential contributions of both physical adhesion and soluble factors, we used three experimental conditions to differentiate these interactions: 1) MM cells cultured in complete medium, 2) MM cells suspended in medium conditioned in the prior presence of BMSCs, or 3) MM cells co-cultured with BMSCs in a transwell system.

Results: Using the median effect method of Chou Talalay, we evaluated the combination indices for simultaneous and sequential treatment schedules, and we found that the schedule of administration is important to maximize the synergistic effects. Indeed, schedule-dependent synergistic cytotoxicity was demonstrated for the combination of IMiDs and Pis and a maximal apoptosis consistently observed in IMiDs pre-exposure schedule. The superiority of this schedule was maintained throughout BM microenvironment models. Our data overall suggest that the administration of IMiDs before Pis can improve efficacy. Clinical trials are needed to investigate the most effective schedule, which could be to start the administration of IMiDs a day before Pis to increase cells killing.

Clinical trials are needed to investigate the most effective schedule, which could be to start the administration of IMiDs a day before Pis to increase cells killing.

Myeloma and other monoclonal gammopathies - Clinical

ASSESSMENT OF THE IMPACT OF POST-AUTOLOGOUS STEM CELL TRANSPLANTATION MAINTENANCE THERAPY ON SURVIVAL OUTCOMES IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA IN THE COMMUNITY-BASED CONNECT MM REGISTRY

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Background: Randomized phase 3 clinical trials have shown that maintenance therapy after autologous stem cell transplant (ASCT) can extend time to progression, progression-free survival (PFS), and overall survival (OS) for patients (pts) with newly diagnosed multiple myeloma (NDMM) (Sonneveld, J Clin Oncol, 2012; McCarthy, N Engl J Med, 2012; Attal, N Engl J Med, 2012; Palumbo, N Engl J Med, 2014; Attal, ASCO, 2016). Connect MM is a largely community-based, US prospective observational cohort study designed to characterize diagnosis, treatment patterns and outcomes in pts with NDMM in clinical practice.

Aims: The Connect MM registry was used to assess impact of maintenance therapy on survival outcomes in pts with NDMM receiving ASCT.

Methods: Adult pts with NDMM were eligible to enroll in the registry within 60 days of diagnosis. Pts were enrolled in 2 sequential cohorts and were treated at the clinician’s discretion as per standard of care. Cohort 1 pts receiving induction and ASCT were included in the analysis and characterized into 4 maintenance regimen subgroups: no maintenance, lenalidomide (LEN)-based maintenance, bortezomib (BORT)-based maintenance, and LEN+BORT maintenance. Duration was from 100 days post-ASCT (no maintenance group) or start of maintenance until progressive disease, death, discontinuation, or data cutoff of January 7, 2016. End points were PFS, second PFS, OS, and safety. An exploratory analysis of the impact of baseline characteristics on survival outcomes was performed.

Table 1.

Results: A total of 1493 pts were enrolled in Cohort 1 from Sep 2009 to Dec 2011; 1450 were treated, 81% (n=1173) in a community setting. Of those, 432 (29%) met analysis criteria. Median follow-up was 39.3 months. Median age was 60 y (range, 24-78); 60% were men; and 86% were white. A total of 165 pts did not receive maintenance. Of 267 pts receiving maintenance, 213 (80%) received LEN-based maintenance; 30 (11%) received BORT-based maintenance; and 16 (6%) received LEN+BORT maintenance. Of the maintenance groups, only data from LEN maintenance is presented; small sample sizes in the other maintenance groups limited interpretation. The median treatment duration was 35.2 months for pts who received LEN maintenance and 26.1 months for those who did not receive maintenance. Median PFS was significantly longer for pts who received LEN maintenance vs no maintenance (50.3 months vs 20.8 months; hazard ratio [HR]=0.62 [95% CI: 0.46, 0.82]; P=0.0098; Table). OS was also significantly improved for pts who received LEN maintenance vs no maintenance (HR=0.54 [95% CI: 0.36, 0.83]; P=0.0050). Second PFS (PFS for second-line treatment) was similar for both LEN and no maintenance groups. Exploratory analyses showed generally similar PFS and OS improvements across subgroups (age, ECOG status, International Staging System stage, risk group, and induction regimen). No new safety signals were observed.

Summary/Conclusions: In this observational study, post-ASCT LEN maintenance therapy significantly improved PFS and OS compared to no maintenance. These improvements appeared to be independent of induction regimen. Preliminary analysis of second PFS suggests no adverse impact of maintenance treatment on the efficacy of second-line therapy. These data, from a largely community-based setting, support results from randomized phase 3 trials.
**E1240**

DARATUMUMAB-BASED COMBINATION THERAPIES IN HEAVILY-PRE-TREATED PATIENTS WITH RELAPSED AND/OR REFRACTORY MULTIPLE MYELOMA

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**Background:** Daratumumab-based combination therapies (DCT) with bortezomib (V)/ lenalidomide (R)/ pomalidomide (P) and dexamethasone (d) have shown exceptional activity in relapsed and/or refractory multiple myeloma (RRMM) in trials. Experience outside of trials since the approval of Daratumumab (D) in 2015 is limited.

**Aims:** We aimed to review the outcomes of patients who received DCT at our institution.

**Methods:** Records of RRMM patients seen at Mayo Clinic, MN from December 2015–December 2016 were reviewed. Patients who received ≥ 1 cycle of DCT were included. Time-to-event analyses were done from date of starting DCT using Kaplan Meier method. Common terminology criteria for adverse events v4.0 were used to grade toxicities.

**Results:** Of 130 patients, 59% were males and median age at DCT initiation was 67 (43-93) years. ECOG performance score was ≤ 2 in 29%. Patients were classified as mSMART high (22%), intermediate (22%) or standard (56%) risk. Median time from diagnosis to initiation of DCT was 51.3 (5-156) months, and median number of prior therapies was 4 (1-14). Eighteen (14%) of patients were refractory to prior daratumumab monotherapy. Fifty-three (41%), 34 (26%) and 25 (19%) received DPd, DRd and DVd respectively. Eighteen (14%) patients received 'other' DCT. Median time to first response (≥ PR) was 3.1 months (95% CI: 2.1-4.6). Overall response rate was 46%, [complete remission-2%, very good partial remission-18%, partial remission-26%]. Minimal remission was seen in 17%, with clinical benefit rate of 62%. Median estimated follow up from initiation of DCT was 5.5 months (CI 4.2-5.7). The median duration of response was 6.1 months [CI 5.1- not reached (NR)]. Median progression free survival (PFS) was 5.5 months (CI 4.1-7.8) (figure A) and median time to next therapy (TTNT) was 5.9 months (CI 4.6-9.4) (figure B). Median PFS for DPd, DRd, DVd and other DCTs were 4.6 (CI 2.7-NR), 7.8 (CI 5-NR), 3.9 (CI 2.1-NR) and 3.9 (CI 2.8-8.2) months, respectively (p=0.3). Median overall survival (OS) from starting DCT was NR (CI 11.4-NR) (figure C). Median PFS for quadruple refractory (n=28) MM was 2.8 months (CI 2.2-5.3) vs 5.9 months (CI 4.9-NR) for the rest (p=0.008) (figure D). Grade 3 or higher hematological toxicities were seen in 42% of patients. Other toxicities included infections (37%), fatigue (31%), infusions reactions (16%) and diarrhea (10%).

**Figure 1.**

**Summary/Conclusions:** DCT are effective in RRMM, but the PFS remains short, particularly in quadruple refractory patients, reflecting the challenges encountered in managing heavily-petreated, and often less fit patients, in routine practice.

**E1241**

IMPACT OF METFORMIN USE IN THE OUTCOMES OF MULTIPLE MYELOMA PATIENTS POST STEM CELL TRANSPLANT

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**Background:** Multiple myeloma (MM), a monoclonal plasma cell disorder, is one of the common hematologic malignancies in the US. In preclinical studies, metformin demonstrated plasma cells cytotoxicity. However there is lack of studies translating the effect of metformin into the clinical setting.

**Aims:** Assess the clinical effect of metformin in patients with MM.

**Methods:** All MM patients who underwent stem cell transplant (SCT) at the Mayo Clinic Rochester from 2007 to 2012 were reviewed. Patients were grouped based on metformin use. Initial diagnosis at our institution and ≥12 months of follow up were required. Kaplan-Meier method and Cox regression were used for time-to-event and multivariate analysis.

**Results:** Out of 687 patients, 78 (11.4%) patients were using metformin at the time of MM diagnosis. Baseline characteristics in the metformin and no-metformin groups were similar. Median metformin dose was 2000mg daily and median duration of metformin use from MM diagnosis was 22 months. Patients on the Metformin group achieved higher rates of complete response after SCT (41% vs 29% p<0.02). Median progression-free survival (PFS) after SCT was longer in the Metformin group, 31.3 months (95% CI: 10.4-52.2) vs 16.6 months in the no-metformin group (95%CI: 14.5-18.7) p<0.04. There was a trend toward longer overall survival in the Metformin group, but it was not statistically significant (170 vs 106 months, p<0.10). In a multivariate analysis of metformin use, age, sex, international staging system (ISS), LDH and cytogenetics/FISH, the former was an independent predictor of PFS after SCT (OR: 0.38, 95%CI: 0.20-0.80, p<0.001).

**Figure 1.**

**Summary/Conclusions:** Metformin use was associated with a better progression-free survival and higher complete response rates after SCT in our MM cohort. A trend toward better overall survival was also noted in the Metformin group. Larger studies are needed to enhance our understanding of the clinic effect of metformin on MM.

**E1242**

COMPARING WHOLE BODY MRI WITH PET-CT IMAGING AT DIAGNOSIS OF MYELOMA

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**Background:** Imaging in the diagnosis of myeloma is a rapidly developing field. First line imaging has traditionally been a skeletal survey with plain films, however new guidelines recommend whole body imaging to aid the diagnosis of myeloma. The International Myeloma Working Group recommend low-dose whole body computerised tomography (LDWBCT), PET-CT or whole body magnetic resonance imaging (WBMRI) as initial imaging modalities.

**Aims:** To compare WBMRI with PET-CT as initial imaging modalities at diagnosis of myeloma or plasma myeloma.

**Methods:** Both WBMRI and PET-CT were performed at diagnosis of myeloma or a plasmacytoma in 33 patients presenting to King’s College Hospital, London. The scans were reviewed independently by two Consultants in Radiology
and Nuclear Medicine, looking for focal bone lesions, bone marrow pattern and incidental findings. Details of the patients’ demographics, myeloma diagnosis and treatment were collected from the medical records.

**Results:** Of the 33 patients, 24 were male. The median age was 64 years (range=43-86 years). One patient had a solitary plasmacytoma, the other 32 had myeloma (21 IgG, 3 IgA, 2 non-secretory, 4 light chain disease, 2 biclonal myeloma, and 1 with ISS stage 1 disease with a median ISS score of 17 (range 0-52.6). 21 patients had a bone marrow plasma cell burden of 10-60%, 10 patients >60% and 2 were unknown. Sixteen patients were diagnosed with smouldering myeloma and a ‘watch and wait’ policy was adopted. Eleven patients were treated with chemotherapy. 4 were entered into a clinical trial, one was offered palliative care and one was referred to our centre for autograft. WBMRI identified a focal lesion of disease in 30% of patients compared with 36% by PET-CT. This was not a statistically significant difference (p=0.18). In addition there was no statistically significant difference between PET-CT & MRI in detecting <3 or >3 lesions (p=0.705 and p=0.083 respectively). The apparent diffusion coefficient at vertebrae L5 (using diffusion weighted MR imaging) was measured. This showed a strong correlation with the degree of bone marrow infiltration by plasma cells (r=0.64). An ADC of <600mm2/s had a negative predictive value of 93% for a bone marrow plasma cell infiltrate of >60%. There was also a significant difference (p=0.012) in the ADC between those with smouldering myeloma and those with symptomatic disease. It was noted that 9 scans resulted in incidental findings including pneumonia, adrenal lesions and one case of coloecal cancer.

**Summary/Conclusions:** We have shown no difference in PET-CT and WBMRI in detecting a myeloma defining focal bone lesion, or providing prognostic estimations of burden of disease. Using MRI, a measure of the ADC at vertebrae L5 has been shown to be a semi-quantitative parameter that correlates with bone marrow plasma cell infiltration and distinguished between those with smouldering and symptomatic disease. In addition it is noted that whole body imaging has led to incidental findings of further pathology, including an unrelated malignancy, which may lead to useful clinical information or to further investigations and imaging which may not be needed.

**E1243**

**PERSISTENCE OF MINIMAL RESIDUAL DISEASE BY MULTIPARAMETER FLOW CYTOMETRY CAN HINDER RECOVERY OF ORGAN DAMAGE IN PATIENTS WITH AL AMYLOIDOSIS**

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**Background:** In multiple myeloma, Minimal Residual Disease (MRD) demonstrated by multiparameter flow cytometry (MFC) identifies subjects with significantly shorter survival compared among those who attain complete response (CR). The role of MRD in AL amyloidosis has not been assessed so far.

**Aims:** In the present study, we assessed the MRD by MFC in patients with AL amyloidosis who attained CR.

**Methods:** CR was defined as per current criteria (negative serum and urine immunofixation, normal light chain ratio). For flow cytometry at diagnosis, bone marrow samples were processed following the Euro Flow Bulk Lysis Standard Operating Protocol and stained with the EuroFlow/MF MM MRD panel. At least 5x106 events were measured using a FACSCanto II (USA) instrument. Data were analyzed using the Infinicyt software (Spain). Patients were identified as having residual disease if a discrete population of plasma cells comprising ≥50 events was identified (10-5 limit of detection).

**Results:** Twenty-eight patients were tested (7 found to have relapsed at the time of MRD assessment with monoclonal components detectable and MRD+ and 21 satisfied current criteria for CR. Nineteen (90%) had renal and 9 (49%) had cardiovascular involvement at diagnosis. More than 2 lines of therapy were required to achieve CR in 7 subjects. Median time to CR was 10 months (range: 3-82). Five patients (62%) had achieved cardiac response and 9 (50%) renal response at the time of CR. The median time from CR to MRD was 30 months (range: 6-148), this was not different in the MRD positive vs negative patients. Reduction in serum free light chain level rate (43%). A median of 1089 (range 256-2500) corresponding to 0.04% (range 0.02-0.3%) plasma cells with abnormal phenotype were detected in patients MRD+. No differences in organ involvement, cardiac and renal stage, type of therapy, number of treatments, and organ response at the time of CR was found between the two groups. Improvement of cardiac or renal function after CR was significantly associated with MRD+ at diagnosis (P=0.04) vs MRD- (P=0.153).

**Summary/Conclusions:** This proof-of-concept study indicates that 43% of patients with AL satisfying current criteria for CR have detectable MRD. MRD positivity could in part explain persistence of organ damage is patients in CR.

E1244

**RATES OF PERIPHERAL NEUROPATHY (PN) IN PATIENTS WITH RELAPSED AND REFRACTORY MULTIPLE MYELOMA (RRMM) TREATED WITH CARFILZOMIB VS COMPARATORS IN PIVOTAL PHASE 3 TRIALS**

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**Background:** High dose chemotherapy followed by autologous stem cell transplant (ASCT) remains the gold standard treatment in myeloma for young patients in CR. A validation study in a larger cohort is ongoing. The possible impact of MRD should be considered in trials aiming at increasing organ response rate in patients in CR.

**Methods:** This proof-of-concept study indicates that 43% of patients with AL satisfying current criteria for CR have detectable MRD. MRD positivity could in part explain persistence of organ damage is patients in CR.

**Summary/Conclusions:** In ENDEAVOR, the rate of PN was significantly lower with Kd vs Vd in patients with baseline history of PN (patients with ≥3 PN at baseline or grade 2 PN at pain with baseline were excluded from the studies).

**Table 1.**

<table>
<thead>
<tr>
<th>Pain in the extremities (≥4)</th>
<th>Pain in the extremities (≥3)</th>
<th>Pain in the extremities (≥2)</th>
<th>Pain in the extremities (≥1)</th>
<th>Pain in the extremities (≥0)</th>
</tr>
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<tbody>
<tr>
<td>Kd (0.0% [95% CI: 0.0-0.0])</td>
<td>0.0 (0.0-0.0)</td>
<td>0.0 (0.0-0.0)</td>
<td>0.0 (0.0-0.0)</td>
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<tr>
<td>Vd (4.0% [95% CI: 2.0-6.0])</td>
<td>4.0 (2.0-6.0)</td>
<td>4.0 (2.0-6.0)</td>
<td>4.0 (2.0-6.0)</td>
<td>4.0 (2.0-6.0)</td>
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**Results:** In ASPIRE, grade ≥2 PN rate was low (8.0% [95% CI: 6.0-10.0%]; Table). Pain subscale scores were similar between arms. Median PFS was longer with KRd vs Rd for patients with grade 2 PN at baseline. In ENDEAVOR, grade ≥2 PN rate during the study (prespecified key secondary endpoint) was significantly lower with Kd vs Vd (6.0% vs 32.0%, Table). Patients had significantly improved pain and neurotoxicity subscale scores with Kd vs Vd. PFS improved with Kd vs Vd in patients with baseline history of grade ≥2 PN (Table 1).

**E1245**

**EARLY RELAPSE FOLLOWING AUTOLOGOUS STEM CELL TRANSPLANTATION IN MYELOMA IS A POOR PROGNOSTIC MARKER FOR OVERALL SURVIVAL AND IS DIFFICULT TO PREDICT AT DIAGNOSIS OR DURING INDUCTION TREATMENT**

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**Methods:** This proof-of-concept study indicates that 43% of patients with AL satisfying current criteria for CR have detectable MRD. MRD positivity could in part explain persistence of organ damage is patients in CR.
Results: Persistent fatigue was the main indication for treatment in 22/31 (71%) pts. Baseline PRO scores were lower for time sub-study vs randomized pts (Table). With a median of 17 months (mo) of treatment, most pts had clinically meaningful improvement in TS (>7 points; 77%), AS (≥6 points; 84%), and EQ utility scores (≥0.08 points; 88%). Time to clinically meaningful improvement was prompt (1 mo for TS and AS; 2 mo for EQ), corresponding with a 48% decline in median IgM (median 20 g/L) after 4 weeks. In pts with baseline anemia (hemoglobin [Hb] <110 g/L), sustained Hb improvement increased with depth of response. At week 65, Hb levels significantly correlated with TS (r = 0.507, P = 0.01) and AS (r = 0.519, P = 0.008), and were marginal for EQ (r = 0.39, P = 0.054). Although IgM levels did not significantly correlate with PRO scores, the benefit was similar in responders regardless of depth of response. 

Table 1.

Summary/Conclusions: Clinical response, and associated anemia improvement induced by ibr, correlated with meaningful improvements in the well-being of heavily pretreated pts with RTX-refractory WM.

E1247

INCIDENCE AND RISK FACTORS OF CARDIOVASCULAR ADVERSE EVENTS IN A LARGE POPULATION OF NEWLY-DIAGNOSED, TRANSPLANT INELIGIBLE MYELOMA PATIENTS TREATED WITH CARFILZOMIB


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Background: Cardio-vascular (CV) toxicities in patients (pts) with multiple myeloma (MM) may derive from comorbidities, MM itself and its treatment. Carfilzomib, an irreversible proteasome inhibitor, is approved as single agent or in combination with dexamethasone or lenalidomide-dexamethasone for relapsed MM.

Aims: We conducted an integrated analysis of CV adverse events (AE) in newly diagnosed, transplant-ineligible MM patients treated with Carfilzomib in 3 phase III studies (IST-CAR-506, IST-CAR-561, IST-CAR-601).

Methods: All pts were treated with 9, 28-day induction cycles with carfilzomib, cyclophosphamide (300mg/m² on days 1,8,15) and dexamethasone (40mg weekly) (CCyC), followed by carfilzomib maintenance until progression or intol-
erance. Carfilzomib was administered i.v. at the dose of 36mg/m² on days 1, 2, 8, 9, 15, 16 in the IST-CAR-506 trial; at 3 dose levels escalated from 45 to 70mg/m² on days 1, 8, 15 in the IST-CAR-561 trial and on days 1, 2, 8, 9, 15, 16 in the IST-CAR-601 trial. AEs were graded based on NCI-CTCAE v4. Results: 148 pts with a median age of 72 years were analyzed. At enrollment, 34% of patients had at least 1 cardiovascular risk factor; 20% had peripheral vascular disease (including hypertension in 13% patients), 19% diabetes and 5% chronic pulmonary disease. After a median follow-up of 21 months, at least 1 any grade CV-AE occurred in 45% of patients; any grade hypertension was reported in 17% of patients, dyspnea in 9%, and heart failure, arrhythmia and venous thromboembolism (VTE) in 6% of patients, each. Grade 3-5 CV-AEs occurred in 12% of patients, the most common being heart failure (4%), hypertension (3%), pulmonary edema (3%) and VTE (3%). Four (3%) fatal CV-AEs occurred: 1 case of heart failure, pulmonary edema, arrhythmia and VTE, respectively. No difference in terms of CV-AEs was observed in patients treated with different doses of carfilzomib. In pts who developed at least 1 CV-AE, carfilzomib dose reduction (33%) and discontinuation (33%) were more frequent as compared to those without CV-AEs (12% and 18%, respectively; p<0.0001). A trend toward a shorter 2-year overall survival (adjusted for age) was observed among patients who experienced at least 1 CV-AE as compared with those who did not (74% vs 83%; HR: 0.51; p=0.066). Pts ≥75 years had a higher risk of any grade (58% vs 36%; p=0.02) and grade 3-5 CV-AEs (34% vs 15%; p=0.01); major cardiac events of any grade were more frequent in older patients (29%) than in younger ones (6%; p<0.001). Patients with at least 1 CV risk factor at enrolment had a 4-fold increased risk (odds ratio: 3.79; p<0.001) of developing a CV-AE during treatment as compared to patients with no CV risk factors; in detail, baseline hypertension (odds ratio: 4.12; p=0.012) and peripheral vascular disease (odds ratio: 3.75; p=0.002) conferred the highest risk of developing CV-AEs.

**Figure 1.**

**Summary/Conclusions:** Among newly diagnosed MM pts treated with carfilzomib, cyclophosphamide and dexamethasone, at least 1 CV-AE occurred in 45% of pts, hypertension and dyspnea were the most common. Pts ≥75 years of age and those with at least 1 pre-existing CV risk factor were at higher risk of developing a CV-AEs. The onset of CV toxicity significantly increased the rate of dose reductions and treatment discontinuation, translating into higher risk of death. CV toxicity may significantly impact on treatment compliance and survival. Therefore, to derive maximum benefit from Carfilzomib, all pts - particularly the elderly - should be carefully assessed to select the most appropriate treatment.

**E1249**

**POMALIDOMIDE (POM) + LOW-DOSE DEXAMETHASONE (LODEX) AFTER SECOND- LINE LENALIDOMIDE (LEN)-BASED TREATMENT OF RELAPSED OR REFRACTORY MULTIPLE MYELOMA (RRMM): UPDATED PROGRESSION-FREE SURVIVAL ANALYSIS**


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**Background:** Most recent pivotal trials of triple therapy in second- and third-line treatment excluded patients (pts) whose multiple myeloma (MM) was refractory to LEN. This is not reflective of the standard of care in first and second line where LEN is given until progressive disease (PD). To address this, the MM-014 phase 2 trial enrolled pts with RRMM and second-line LEN-based treatment failure. Cohort A enrolled pts treated with POM + LoDEX. The study was amended to include cohort B (pts treated with POM + LoDEX + duratumab).

**Aims:** To present updated safety and efficacy analyses only from cohort A, in which pts received POM + LoDEX immediately after relapsing or being refractory to second-line LEN-based therapy.

**Methods:** Pts aged ≥18 years had documented MM, measurable disease, 2 prior lines of treatment, and PD after ≥2 cycles of second-line LEN-based treatment. Pts received 28-day cycles of POM 4mg/day on days 1-21 + LoDEX 40mg/d (days 1-21, days 1-15) or 40mg/d (days 1-7, days 1-15) until progression or PD, or until a maximum of 3 cycles of carfilzomib was mandatory. The primary endpoint was overall response rate (ORR; ≥ partial response [PR]) assessed by modified IMWG criteria. Key secondary endpoints included time to response (TTR), progression-free survival (PFS), secondary primary malignancies (SPMs), and biomarkers. All pts provided informed consent.

**Results:** Of 51 enrolled pts in cohort A, 39 (76.5%) discontinued treatment, mostly due to PD. Median age was 68.0 years, and 92.2% had an Eastern Cooperative Oncology Group performance status of ≤1. A total of 45 pts (88.2%) were refractory to their last treatment with LEN, and 37 (72.5%) had prior treatment with pomalidomide. Median duration of prior LEN-containing therapy was 24.6 months. With a median follow-up of 13.6 months, ORR was 29.4%, with 1 (2.0%) complete response, 5 (9.8%) very good partial responses, and 9 (17.6%) PRs. Minimal response (MR) was reached in 15.7% of pts. Median TTR was 1.9 months and 66% of pts had ongoing response at 1 year. Median PFS was 13.8 months. The 2-year PFS rate was 48.6% for the intent to treat population. 69.4% for pts with ≥ MR, and 69.1% for pts with ≥ PR. In addition, pts with ≥ MR had similar treatment durations as those achieving ≥ PR (10.5 vs 11.5 months; Table). Complete grade 3/4 adverse events (AEs) included anemia (25.5%), neutropenia (11.8%), and infections (19.6%); including pneumonia (9.8%). No pts experienced SPMs. In the immune subset analysis, the proportions of CD3+ and CD3+CD8+ T cells after treatment were significantly higher vs baseline (72.6% vs 67.8% and 36.9% vs 32.1%, respectively; P<0.05).

Pts with response also had significantly elevated proportions of these T-cell populations, but pts with no response did not. Relative changes from baseline for CD3+ and CD3+CD8+ T-cell populations were significantly greater in pts with response vs those with no response (10.4 vs -0.8 and 4.2 vs -3.5, respectively; P<0.05).

**Table 1.**

**Summary/Conclusions:** This update confirms the safety and efficacy of POM + LoDEX following second-line LEN-based treatment failure in pts with RRMM. Hematologic AE rates improved, and median PFS was longer with third-line use than previously reported with POM + LoDEX use in later treatment lines. In addition, achieving disease control of ≥ MR led to similar PFS rates as reaching ≥ PR.

**E1249**

**“REAL WORLD” DATA ON THE EFFICACY AND SAFETY OF IZAXOMIB IN COMBINATION WITH POMALIDOMIDE AND DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA: A STUDY OF THE GREEK MYELOMA STUDY GROUP**

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Madrid, Spain, June 22 – 25, 2017
and 4 with other therapies. Baseline characteristics were balanced across who were on treatment, 63 were treated with LEN, 6 with BORT, 0 with THAL, for BORT, and 4.6 mos (range 0.2-36.9 mos) for THAL. At the time of analysis, (range 0.1-79.9 mos) for patients receiving LEN, 4.1 mos (range 0-61.4 mos) for BORT, and 4.6 mos (range 0.1-79.9 mos) for patients receiving THAL.

Results: Of these patients, 2151 (59.3%) received LEN, 1187 (32.7%) received National Cancer Institute-Common Terminology Criteria for AEs, v3.0. SPMs prior therapy initiating a non–LEN-based treatment were enrolled into a back-investigator’s discretion into the LEN cohort (LEN + dexamethasone, the prior therapy was a partial bortezomib responder, 7 and who had at least two separate but related objective responses, 0 to 6 mos for 11/34, MR in 2.9% (1/34) and stable disease in 26.5% (9/34). The ORR (PR or better) was 67.6%. 70.8% among those who received IRd in the second line and 60.0% among those who received IRd beyond the second line; ORR tended to be higher as the time of exposure to ixazomib increased, with patients exposed to ixazomib 6-8 weeks (67.7%), 9-12 weeks (62.5%), and 13-16 weeks (59.3%) prior to randomization, respectively. Median time to best response was 1.2 months. Treatment interruptions due to AEs were recorded for 11.4% (4/35) of patients, while 20.0% (7/35) of patients discontinued treatment. Reasons for ixazomib discontinuation were AEs for 3 patients (an event of cardiac arrhythmia or other cardiac events), death (one due to cardiac arrhythmia not resolved, one due to radiation treatment for cardiac events) and disease progression, while 31.4% (11/35) of patients developed gastrointestinal neuropathy; of those events, 54.5% (6/11) resolved, while 45.4% (5/11) not resolved (three were grade 1, one grade 2, and one of grade 3) at the end of follow-up. In addition, 31.4% (11/35) of patients developed gastrointestinal AEs, 11.4% (4/35) experienced pneumonia, 9.4% (3/32) hypertension, 5.7% (2/35) cataract and herpes zoster, and 2.9% (1/35) deep vein thrombosis; no cardiac arrhythmia or other cardiac events were observed, while osteonecrosis of the jaw developed in 5.7% (2/35) of the patients.

Summary/Conclusions: This study showed that the IRd regimen produces an ORR of near 68% and a clinical benefit in almost all patients with RRMM who are treated in IRW practice. IRd acts rapidly and has an acceptable toxicity profile with no cardiac events.
Results: During the development process, a number of similarities and discrepancies between centers as well as evidence gaps were identified. Intense discussion and literature searches resulted in a concise, harmonized clinical pathway, released by all 14 Centers of Excellence. This is freely available on the website ccc-netzwerk.de and provides a very decisive insight according to the current state of knowledge on the CCC-level (e.g. on the diagnostic algorithm Fig. 1). The clinical pathway is well suited for informing patients and physicians about the most up-to-date, comprehensive medical treatment standards as well as innovative procedures. Furthermore, this project initiated the idea of developing a national evidence-based clinical practice guideline for MM in the frame of the German Guideline Program in Oncology.

**E1252**

**WT1 HETEROCLLITIC EPITOME IMMUNIZATION FOLLOWING AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH HIGH-RISK MULTIPLE MYELOMA (MM)**

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**Background:** The Wilms tumor 1 (WT1) protein is a tumor associated antigen that is a target for anticancer immunotherapy. We had previously demonstrated overexpression of WT1 in multiple myeloma (MM) cells by IHC, as well as formation of a WT1 peptide fragment (RMFPNAPYL)/HLA-A*0201 complex on the engagement interface between malignant plasma cells and T-cells in HLA-A*0201 MM pts using the high-affinity fully human IgG1 mAb ESK1. We report initial results from MM pts immunized with the WT1 heteroclitic peptide mixture galinepepimut-S (GPS) after autoSCT.

**Aims:** To determine the safety and potential efficacy of the WT1 heteroclitic peptide immunizer GPS administered in patients with multiple myeloma following autologous stem cell transplantation.

**Methods:** 16 MM pts underwent autoSCT with melphalan conditioning followed by (f8b) lenalidomide maintenance starting 3 months (mos) post-SCT. 13/16 pts presented with high-risk (HR) cytogenetics [t(4;14), 1(4;16), del17p, 1q21q25 gain and/or del(13q)]. GPS was administered with montanide s.c. starting 2 ws post-SCT and q2 ws thereafter x 6 initial doses f8b boosters q4 ws x 6 additional doses. GM-CSF was given on days -2 and 0 of each cycle.

**Results:** 16 pts underwent auto SCT followed by WT1 heteroclitic peptide immunization; median follow-up of 18 mos (range: 5-31 mos) for survivors; median age: 61.5 y. Overall survival (OS) and progression-free survival (PFS) (95% CI) at 18 mos: 0.88 (0.73-0.99) and 0.62 (0.42-0.97) respectively. Current median PFS: 23.6 mos (15.2- not reached). No ≥G2 systemic side effects were observed, however, all pts developed local nodularity at the site of injections which resolved over 2 – 6 ws. Both CD8+ and CD4+ IRs could be detected at various levels and were induced not only against the heteroclitic peptides (within GPS), but also against the corresponding native WT1 peptide sequences as well as the ‘total pool’ of WT1-derived overlapping peptides.

**Summary/Conclusions:** Administration of the novel WT1 heteroclitic peptide immunizer GPS post-SCT demonstrates favorable safety profile along with an encouraging mPFS of currently 23.6 mos in this high-risk MM population. This trial suggests WT1 heteroclitic peptide therapy is easy to administer and has been specifically designed to elicit responses across most common HLA Class I and II alleles. Based on these results, a larger phase II trial is being planned to optimally integrate post-transplant immunotherapeutic strategies to meaningfully delay or reduce risk of relapse in this challenging clinical setting.

**E1253**

**ANALYSIS OF MULTIPLE MYELOMA PATIENTS WITH PROGRESSIVE DISEASE AT TIME OF FIRST AUTOLOGOUS STEM CELL TRANSPLANTATION: PREDICTORS OF PROGRESSIVE DISEASE AND FACTORS AFFECTING SURVIVAL**

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**Background:** The impact of response depth at time of autologous stem cell transplantation (ASCT) on the progression-free and overall survival (PFS and OS, respectively) of multiple myeloma (MM) patients has been an extensively investigated area. Rosiñol et al. (2011) reported a significantly worse PFS and OS in patients with progressive disease (PD) compared to stable disease (SD) at time of ASCT; Parrish et al. (2015) stated a significantly shorter PFS, but not OS, in patients with PD in comparison to SD or minimal response (MR) at time of ASCT.

**Aims:** Our goal was to characterize MM patients with PD at time of first ASCT. We aimed to analyze the effects of PD on PFS and OS. Next, we intended to investigate the influence of PD on factors (evaluated at the beginning of induction therapy) as well as use of novel agents in induction therapy, response after 1st ASCT, and use of maintenance therapy in those patients to identify predictors for OS and OS. Furthermore, we aimed to identify clinical/therapeutic features predicting the occurrence of PD before ASCT.

**Methods:** We included 16 MM pts who had undergone a single or tandem ASCT at the University Hospital Heidelberg in the years 1992-2014 and were analyzed regarding their response before first ASCT (d -100 or, if available, response as close to the date of ASCT as possible, i.e. until d -4). Of the 874 ASCT-patients, 829 were eligible for a PFS- and 832 for an OS-analysis. In 51 patients, PD was present at time of ASCT. PFS and OS of those patients were compared with the survival of patients with at least SD at time of ASCT (non-PD patients). Furthermore, clinical factors at beginning of induction therapy, including age (< vs ≥ 65 years), ISS stage, elevated LDH, use of novel agents in induction therapy, high-risk FISH cytogenetics (at least one of the following: del(17p), 1q21 gain, t(4;14)); response after ASCT, and maintenance therapy (yes vs no) were analyzed regarding their impact on PFS and OS of patients transplanted in PD. We also analyzed clinical factors at beginning of as well as use of novel agents in the induction therapy regarding their impact on the presence of PD before ASCT. Response was evaluated according to EBMT-criteria. PFS was calculated from date of 1st ASCT, except for prognostic impact of response assessment after 1st ASCT, where date of response assessment was used. Start of maintenance therapy was analyzed as time-dependent factor.

**Results:** Non-trial patients transplanted in our center between 1992 and 2014 were analyzed, and 51 patients transplanted in PD ≤100 days before ASCT had similar PFS and OS as non-PD patients. Neither the clinical parameters at induction start, response after 1st ASCT, nor maintenance therapy had a significant effect on PFS in those patients. In the univariate analysis, high-risk cytogenetics as well as elevated LDH at induction start had a significantly negative effect on OS in patients with PD before ASCT (HR= 17.12, p = 0.0017; HR=6.09, p = 0.01, respectively), compared to non-PD patients with no high-risk cytogenetics or with normal LDH. Furthermore, ISS stage III was a significant predictor (OR= 3.35, p = 0.02)) of occurrence of PD before ASCT.

**Summary/Conclusions:** In conclusion, our analysis of 51 patients with PD at time of ASCT among 874 ASCT-patients with MM transplanted between 1992 and 2014 shows no significant difference regarding PFS and OS between patients with PD and other response rates. It was further shown that high-risk cytogenetics as well as elevated LDH at beginning of induction therapy have a significant impact on worse OS in patients with PD at time of ASCT. In addition, ISS stage III is a significant factor for occurrence of PD before ASCT. The impact response depth at time of ASCT is not entirely clear, especially regarding the benefit of ASCT in patients with PD at time of ASCT, as reports from other centers show significantly worse PFS and OS (Rosiñol et al.) or only PFS (Parrish et al.) in patients with PD at time of ASCT.

**E1254**

**SEVERE INFECTIONS IMPACTS OVERALL SURVIVAL IN ACTIVE MULTIPLE MYELOMA PATIENTS**

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LENALIDOMIDE PLUS HIGH-DOSE VERSUS LOW-DOSE DEXAMETHASONE FOR THE TREATMENT OF RELAPSED/REFRACTORY MULTIPLE MYELOMA: A SYSTEMATIC REVIEW

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Background: Lenalidomide in combination with dexamethasone is approved globally for the treatment of multiple myeloma (MM). Although older pivotal regimens were used lenalidomide in combination with dexamethasone (LD), more recent studies have used lenalidomide plus low-dose dexamethasone (LD) for relapsed/refractory MM (RRMM), as the LD regimen demonstrated better survival with lower toxicity in the treatment of newly diagnosed MM.

Figure 1.

Summary/Conclusions: PI+IMiDs may be associated with incremental occurrence of specific CV events during treatment, and may result in specific CV events earlier during therapy than PIs or IMiDs alone. These highlight a need for treatments that do not exacerbate CV risks and are appropriate for patients with pre-existing CV conditions. The lower prevalence of baseline CV comorbidities and lower mean age in patients on PI+IMiDs suggest that prevalence of a CV comorbidity and age influences treatment choice. Further analysis may be necessary to better understand the impact of baseline CV comorbidities on choice of MM treatment.

E1256

LENALIDOMIDE PLUS HIGH-DOSE VERSUS LOW-DOSE DEXAMETHASONE FOR THE TREATMENT OF RELAPSED/REFRACTORY MULTIPLE MYELOMA: A SYSTEMATIC REVIEW

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Background: Lenalidomide in combination with dexamethasone is approved globally for the treatment of multiple myeloma (MM). Although older pivotal regimens were used lenalidomide in combination with dexamethasone (LD), more recent studies have used lenalidomide plus low-dose dexamethasone (LD) for relapsed/refractory MM (RRMM), as the LD regimen demonstrated better survival with lower toxicity in the treatment of newly diagnosed MM.
Methods: We collected the clinical data of 169 patients qualified to RRM patient characteristics similar to ELOQUENT-2 were eligible to ensure comparability. Studies with a follow-up of 16–25 months were evaluated separately from studies with a follow-up of >30 months; these observation periods approximately align with those of ELOQUENT-2.

Results: From an initial bibliographic search yielding 5155 non-duplicate results and 619 registry results, 7 studies (8 publications) met the inclusion criteria (4 LD studies, 3 LD studies). Data for overall survival and tolerability from 1153 patients in the LD group and 353 patients in the LD group were analyzed. The median patient age was 63–88 years. Most patients were white, male and had an ECOG score of 0. LD was not associated with loss of efficacy in terms of overall survival; after >30 months of follow-up, the hazard ratio for LD vs VD was 1.04 (95% CI 0.85–1.28). Tolerability was similar for LD vs VD; after 16–25 months of follow-up, LD was associated with a statistically significantly increased risk of Grade 3/4 adverse events (AEs; relative risk [RR]: 1.10 [95% CI 1.01–1.18]). However, after >30 months of follow-up, LD was not associated with statistically significantly increased risk of Grade 3/4 adverse events (RR: 1.00 [95% CI 0.90–1.10]).

Summary/Conclusions: Overall survival and safety are not significantly affected by different dosages of dexamethasone in combination with lenalidomide; thus, use of LD seems to be reasonable in this patient population. Further studies may provide additional evidence to inform clinicians and revision of international guidelines for dexamethasone dosing in RRM.

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E1257

HIGH EFFICACY AND SAFETY OF VTD AS AN INDUCTION PROTOCOL IN NEWLY DIAGNOSED MM PATIENTS ELIGIBLE FOR HDT/AUTOSCT – A REPORT OF POLISH MULTIPLE MYELOMA STUDY GROUP

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Background: Three drug bortezomib-based regimens are nowadays generally recommended standard induction therapy for transplant-eligible patients with newly diagnosed multiple myeloma (MM). The choice between different regimens depends on drug availability in particular countries, their toxicity profile and local preferences. Observations from routine practice might have though significant clinical impact. Aims: The aim of this retrospective analysis was to evaluate the efficacy and safety of VTD regimen in newly diagnosed MM patients eligible for HDT/autoSCT in routine clinical practice.

Methods: We collected the clinical data of 169 patients qualified to HD/MM and transplanted as induction therapy at 4 transplant centers in Poland.

Results: In the cohort of 169 patients, median age was 59 years (range 36-70). ISS stage I was found in 30.8% of patients, ISS stage II in 39.5% and III in 29.7%, respectively. Median number of VTD cycles was 5. In 81.6% of patients bortezomib was administered subcutaneously. Thalidomide dose was 100mg a day in 85.1% of patients. Bortezomib dose was reduced in 43 patients (25.4%) with peripheral neuropathy as the most common reason (75%). Neupropathy was also the most common grade 3/4 adverse event, observed in 20 patients (11.8%) and neuropenia was the most common hematologic toxicity, though it was noted only in 5 patients (3%). Response rate ≥ PR was achieved in 95% of patients, including 5.6% of SCR, 27.1% of CR and 35.1% of VGPR. So far, stem cell mobilization was performed in 110 patients, most commonly used protocols were cyclophosphamide (42.9%), busulfan (16.2%) and a combination of busulfan and filgrastim. Thalidomide dose was 100mg a day (days 1-21), dexamethasone 20mg a day (days 1-2, 4-5, 8, 9, 11, 12) or 40mg a day (days 1-4), every 21 days. Patients were included into analysis if ≥1 cycle of VTD was administered. Adverse events (AEs) were graded according to CTCAE v4.0. The analysis involved also the impact of VTD regimen on efficiency of stem cells mobilization as well as high dose therapy/autologous stem cell transplantation (HDT/autoSCT) procedure.

Summary/Conclusions: In dialytic protocol as conditioning regimen in 77.6% of patients. Median number of transplanted CD34+ cells was 4.4 x 10^6/kg. Median time to reach ANC count ≥ 0.5 G/L and PLT count ≥ 20 G/L was 11 days and 12 days, respectively. In the evaluation of response 100 days after HDT/autoSCT was performed in 81 patients, SCR rate increased from 5.6% to 12.7% and CR from 27.1% to 36.7%.

E1258

HIGH CUT OFF HEMODIALYSIS FOR RENAL RECOVERY IN PATIENTS WITH MULTIPLE MYELOMA: FIVE YEARS OF EXPERIENCE

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Results: The patients were 12 men and 7 women, aged 60±4 years (37–73 years). 10 patients were diagnosed with lambda FLC MM and 9 with kappa type. A total of 244 sessions were conducted, with an average of 11.6 sessions per patient (range 3–27). In all cases reduction of serum FLCs concentration was successfully achieved (90.9% reduction). At the end of treatment with HCO-HD, the reduction of lambda and kappa FLCs concentrations was 85% and 94%, respectively. The average reduction per dialysis session was 65% for lambda and 60% for kappa. 17 out of the 21 treated cases recovered sufficient renal function to become independent of dialysis (80.9% renal recovery).

Summary/Conclusions: In dialysis-dependent AKI secondary to MM, combination HCO HD with chemotherapy allows a sustained reduction of FLCs levels, representing an effective therapy in renal recovery.

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IMPACT OF IMMUNOPAREISIS IN PATIENTS WITH LIGHT CHAIN AMYLOIDOsis

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Background: The presence of immunoparesis (IP) at diagnosis in several plasma cell disorders is a risk factor for progression, associated with an unfavorable outcome with reduced progression-free survival (PFS) and overall survival (OS). However, its impact in light chain (AL) amyloidosis has been evaluated only in few series, and when present it was associated with worst response and survival.

Aims: The aim of this study was to investigate the prognostic impact of IP in patients with newly diagnosed AL amyloidosis at a single institution.

Methods: We reviewed the clinical records of patients with AL amyloidosis diagnosed from January 2008 to December 2016. Sixty-nine patients (32F/37M; median age at diagnosis 62) with available immunoglobulin (Ig) measurements were the final study population. Initial baseline demographics, clinical and laboratory data, treatment and follow-up were collected. Median follow-up was 30.2 months. IP was defined as suppression of all uninvolved Ig below the lower reference value. PFS and OS were calculated from the date of diagnosis.

Results: Forty-three patients (62.3%) were transplant ineligible while 26 (37.7%) underwent an autologous stem cell transplantation (ASCT). The distribution of the monoclonal protein isoform by immunofixation at diagnosis was as follows: light chains only (46.4%), IgG (39.1%), IgA (10.2%) and IgM (4.3%). The predominant light chain isotype was lambda (79.7%). A very good partial response (VPR; 37.7%) underwent an autologous stem cell transplantation (ASCT). The dis-

PFS and OS were calculated from the date of diagnosis.

Summary/Conclusions: Among patients with newly diagnosed AL amyloidosis at a single institution, the presence of IP at diagnosis could be an additional powerful discriminatory prognostic marker. The impact of IP in AL amyloidosis on PFS and OS was similar to that observed in MM patients receiving SLT. The presence of IP at diagnosis was associated with worse PFS and OS in Japanese MM patients.

References
REAL-WORLD RESULTS OF DARATUMUMAB MONOTHERAPY IN HEAVILY PRETREATED RELAPSED/REFRACTORY MULTIPLE MYELOMA IN POLAND: A PROSPECTIVE OBSERVATIONAL STUDY OF THE POLISH MYELOMA GROUP

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Summary/Conclusions: In this first real-world analysis we confirm that daratumumab monotherapy is able to induce response in one third of highly pretreated and double refractory RRMM patients. Regarding safety, in contrast to the SIRIUS trial where no treatment discontinuations due to AEs occurred, 3/26 pts (11.5%) had a ECOG performance status score 2 or lower. Data on treatment outcomes and complications were anonymously collected using electronic CRFs. The IMWG response criteria were applied.

Results: In total 30 patients were qualified to DaraCUP in Poland and all were enrolled to the PMG observational study. At the time of writing this report, 26 pts (87%) had received at least one dose of daratumumab and were included in the safety analysis, while 22 pts (73%) had received at least 2 cycles of daratumumab and were included in the preliminary efficacy analysis. Baseline pts characteristics are reported in Table 1. Pts were heavily pretreated, with a median of 4 prior lines of therapy (range, 2-10). Ten pts (38.5%) were double refractory to both PI and IMiD while 15 pts (53%) were refractory to the last line of previous therapy. Median time since initial diagnosis to start of treatment with daratumumab was 3.9 years (range, 1.4-12.2 years). At the time of analysis, the median follow-up time within the study was 5.1 months (range, 0-8 months) and median daratumumab treatment duration was 4.4 months (range, 0-8 months). Sixteen pts (61.5%) remain on treatment, while ten pts (36.5%) discontinued therapy as a result of disease progression (n=7) and adverse events (AEs) (n=3). Overall response rate (CR or better) was 31.8% including one (4.5%) CR and two (9%) VGPR (Table 1). Stable disease was reported in 11 (50%) pts. The median PFS and OS had not been reached. During the time of observation three deaths were recorded due to disease progression. Regarding daratumumab toxicity, grade 3 or 4 non-haematological toxicities occurred in 8 pts (30.7%) and included: infusion-related reactions (n=2), pneumonia (n=2), other infections (n=2), mandible osteoradionecrosis (n=1), neutropenia (n=1) and thrombocytopenia occurred in 3 pts (11.5%). Grade 3 or 4 anaemia and neutropenia were found in 3 (11.5%) and 2 (7.7%) pts, respectively. Updated results will be presented at the meeting.

Table 1.
Background: Multiple myeloma (MM) is the second most common haematological malignancy after non-Hodgkin lymphoma, accounting for 13% of blood malignancies and 1% of all cancers1. The medical management of multiple myeloma has changed over the years and is influenced by multiple factors (e.g., evidence from clinical trials, drug approval status, level of drug reimbursement, guidelines), which vary across Europe. Information describing how patients are managed in the real world is needed.

Aims: The aim of this analysis was to investigate real-world treatment patterns and patient characteristics in MM across Europe.

Methods: Physicians in Europe were requested to answer a series of questions on patient characteristics and treatment regimens of the last eight patients that they had treated during the month prior to answering the questionnaire, according to their patients’ medical charts. The questionnaire was conducted between January and June 2016. Data on 2564 patients with MM were available and are presented here. Countries were grouped into regions according to similar health care systems: Spain, Portugal, Italy and Israel (Southern Region, SR, n=638); Austria, Switzerland, Belgium, Norway, Sweden, Switzerland and Finland (Central and Northern Region, CNR, n=776); Croatia, Estonia, Latvia, Lithuania, Poland, Serbia, Slovakia (Eastern Region, ER, n=689). Analyzes were descriptive.

Results: Patient characteristics were generally similar across regions, with the majority being <75 years old (69-76%), receiving frontline therapy at study inclusion (57-58%), and being ineligible for autologous stem cell transplant (ASCT) (53-59%). The median time from MM diagnosis to the time that the physician answered the questionnaire was higher in ER (19.5 months) than other regions (9.7-11 months) (Table). The majority of frontline regimens contained bortezomib, although this was lower in ER (51%) than in other regions (66-70%). The median duration of frontline therapy was longer in ER (4.5 months) than other regions (3.2 months). This difference was mainly driven by ASCT eligible patients having longer duration of therapy in ER (4.5 months) than other regions (2.2 months). The number of bortezomib injections in frontline therapy, however, was higher in SR and CNR (both 24) than in ER (18). The majority of second line regimens contained lenalidomide (57-64%) in all regions except ER, where bortezombased regimens were most frequent (38%). The median duration of second line therapy was shorter in SR and CNR than in ER (Table). Moreover, second line therapy, ASCT eligible patients had shorter duration of therapy in ER and SR (3.2 months) than in CNR regions (4.5 months). The majority of later-line (3+) regimens were based on therapies that did not include bortezomib, lenalidomide or pomalidomide for all regions (57-67%) with the exception of SR where pomalidomide (29.4%), lenalidomide (12.6%) and bortezomib (14%) were the preferred options. In this analysis conducted in Europe, treatment patterns differed in terms of regimens used and duration of therapies even though patient characteristics were generally similar across regions. This may in part be related to differences in healthcare systems. Further research is required to understand how new therapies can be integrated into current treatment patterns and how these treatment patterns impact patient outcomes.

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Summary/Conclusions: In this analysis conducted in Europe, treatment patterns differed in terms of regimens used and duration of therapies even though patient characteristics were generally similar across regions. This may in part be related to differences in healthcare systems. Further research is required to understand how new therapies can be integrated into current treatment patterns and how these treatment patterns impact patient outcomes.

E1264

FRAILTY AND MORTALITY IN ELDERLY PATIENTS WITH MULTIPLE MYELOMA

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Background: Worldwide, life expectancy continues to rise. The treatment of elderly people with cancer poses special challenges that should be better addressed. Frailty is a geriatric syndrome associated with reduced functional reserve, impairment in multiple physiological systems, and reduced ability to regain physiological homeostasis.

Aims: To evaluate the impact of the level of frailty on early death and overall survival of elderly patients with multiple myeloma.

Methods: Retrospective study of 150 patients older than 65 years with a recent diagnosis of multiple myeloma from January 2006 to December 2012. Patients were treated with IMIDs, alkylating or bortezombased chemotherapy based on physician preference blind to the geriatric assessment. A check list for frailty burden measurement was used based on Edmonton frailty scale and included: cognitive impairment, depressive disorder, polypharmacy, urinary incontinence, functional impairment, gait disturbance or falls, low weight or weight loss and previous hospitalization. Level of frailty was scored as the sum of each area involved. One record of all the variables was obtained from a retrospective review of the centralized and computerized medical records of patients, using predefined standardized criteria. Patients were classified as fit (0-1 frailty criteria), vulnerable (2-3 criteria) or frail (≥ 4 criteria). OS and PFS were estimated using the Kaplan Meier method using Stata13 program. Group differences according to frailty were investigated using the Cox proportional hazard model accounting for ISS, age, Charson comorbidity index and treatment.

Results: From the 150 patients evaluated, 124 patients were included in the study. The median age was 77 years (range 65-98). Thirty one percent of the patients were older than 80 years, 51% were female. The median Charlson Comorbidity index was 2 (range 0-7), 28% had renal failure and 40% of the patients presented with Myeloma ISS 3. Sixty five percent of patients met at least one frailty criteria and 31% of patients were considered frail. The most common findings were polypharmacy, gait and functional impairment. Most patients were treated with IMIDs (47%); alkylating agents (33%) or bortezomib (14%) based chemotherapy. There was no difference in treatment according to frailty group (p=0.38). The median overall survival time was 75 months (95% CI 53-110), 39 months (95% CI 19-64) and 17 months (95% CI 5-37) for fit, vulnerable and frail patients respectively (log rank p 0.0002). Frailty was specially associated with early death [OR 8.2 (95% CI 1.9-34) p=0.0007]. In the multivariate analysis a higher risk of death was observed related to age [ HR 1.07 (95% CI 1.02-1.12) p=0.002], number of frailty criteria [HR 1.13 (95% CI 1.02-1.20) p=0.001], the presence of renal failure [HR 2.6 (95% CI 1.8-3.8) p=0.0011] and history of hospitalization [HR 2.6 (95% CI 1.8-3.8) p=0.0011]. The frailty criteria independently associated with death were incontinence polypharmacy and previous hospital admissions. Frailty was specially associated with early death [OR 6.2 (95% CI 1.9-34) p=0.0007].

Summary/Conclusions: This study shows that the prevalence of frailty syndrome is high and has a profound impact in early death. It is also independently associated with a worse prognosis. Frailty should be considered as part of the clinical assessment when treating elderly patients with myeloma.

E1265

PROGNOSIS OF AL AMYLOIDOSIS WITH KIDNEY INJURY

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Background: AL amyloidosis is a rare disease related to excessive and uncon-14nerved free light chains. The consequence of this proliferation is an alteration of the affected organs due to deposition of free light chains. Despite therapeutic advances in recent years based, among others, on the finding of French studies, the prognosis of this disease remains poor in particular for patients with cardiac disease. Kidney involvement is also frequently observed in the form of a classic renal amyloidosis, but at present the prognosis of chronic renal failure in this context is unknown.

Aims: The study was interested in the prognosis of AL amyloidosis associated with endstage renal disease on dialysis in the era of treatment with bortezomb.

Methods: A total of 133 patients (61 from Ile-de-De-France region register and 72 from reference center) were analyzed. Median survival was 66.7 months compared to 70.6 months for patients without dialysis (p=0.65). Within the group

Table 1.

Summary/Conclusions: This study shows that the prevalence of frailty syndrome is high and has a profound impact in early death. It is also independently associated with a worse prognosis. Frailty should be considered as part of the clinical assessment when treating elderly patients with myeloma.

Table 2: Univariate and Multivariate Cox Regression Analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
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<tbody>
<tr>
<td>HR (95% CI)</td>
<td>P value</td>
<td>HR (95% CI)</td>
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<td>Number of Frailty criteria</td>
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<td>ISS</td>
<td>2.6</td>
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<td>Presence of Renal Failure</td>
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<td>Bortezomb</td>
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<td>(0.45-3.37)</td>
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</tbody>
</table>
of patients on dialysis, there is no significant difference between those receiving or not bortezomib. Median survival before 2008 was 54.82 months and rose to 82.30 months for patients treated after this date (p=0.95). Age (HR: 0.2819, CI 0.1375 to 0.5782), heart disease (HR: 0.3746, CI 0.1724 to 0.8141) and serum albumin (HR: 2.500 CI: 1.077 to 5.803) were identified as prognostic factors. Transplantation is a viable treatment option for good responders.

Summary/Conclusions: Prognosis of AL amyloidosis in dialysis is heterogeneous. Prognostic scoring integrating clinical biological data could identify the patient who may benefit the most dialysis. This results need to be matched by sex and age with non-dialysis and dialysis for another cause.

E1267

FDG-PET IN MULTIPLE MYELOMA: DUAL TIMEPOINT FDG UPTAKE IN FOCAL LESIONS CORRELATES TO RESPONSE TO CHEMOTHERAPY


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Background: Dual Time Point (DTP) 18F-FDG PET imaging has been shown to be useful in differentiating malignant from benign lesions in that increasing uptake from 1 to 3 hours is a characteristic feature of malignancy in contrast to inflammation.

Aims: The aim of this study was to evaluate the predictive role of DTP 18F-FDG PET/CT imaging in assessing response to chemotherapy in multiple myeloma (MM).

Methods: 23 patients with MM (21 male, aged 53-75 years) underwent 18F-FDG PET/CT in a prospective study (NCT02187731) before start of treatment and two months after high dose chemotherapy with stem cell support. All scans were performed at 60 and 180 minutes after tracer injection at Odense University Hospital and Vejle Hospital. Thirteen patients with ≥ 3 focal lesions of at least 10 mm were selected for analysis. Images were analyzed using an adaptive thresholding algorithm (ROVER software, ABX GmbH, Reinbek, Germany). Focal malignant lesions were localized in pre-treatment scans; however, apart from clinical trials, there is limited data for the efficacy of this combination as 2nd line treatment. Furthermore, the efficacy of LenDex when administered before evident clinical manifestations, namely in the case of biochemical relapse as compared to clinical relapse, has not yet been assessed.

Background: The combination of lenalidomide/dexamethasone (LenDex) is an established treatment for relapsed/refractory Multiple Myeloma (MM) patients; however, apart from clinical trials, there is limited data for the efficacy of this combination as 2nd line treatment. Furthermore, the efficacy of LenDex when administered before evident clinical manifestations, namely in the case of biochemical relapse as compared to clinical relapse, has not yet been assessed.

Aims: In the current study, we evaluated response rates and progression-free survival (PFS) in patients treated with LenDex in 2nd line and we compared survival rates of patients treated with LenDex at biochemical relapse vs those treated at clinical relapse.

Methods: Medical files of 207 patients with MM diagnosed between 2000-2013 in 18 Greek centers and treated with LenDex as 2nd line treatment from January 1st 2009, up to March 1st 2014, were retrospectively studied. Overall response and PFS were evaluated for all patients. Additionally, PFS was compared in patients treated at either biochemical relapse (group A) or at clinical relapse (group B). The prognostic significance of biochemical relapse adjusted with important patients' characteristics was also evaluated. Classical methods were used for statistical analysis.

Results: Two hundred and seven patient files were recorded and analyzed (M/F: 112/95, median age: 67.2y, range 31-91y). Overall response and PFS were evaluated for all patients. Additionally, PFS was compared in patients treated at either biochemical relapse (group A) or at clinical relapse (group B). The prognostic significance of biochemical relapse adjusted with important patients' characteristics was also evaluated. Classical methods were used for statistical analysis.

E1268

UNDERSTANDING THE CONTRIBUTE OF THE NOTCH PATHWAY IN MULTIPLE MYELOMA BONE MARROW NICHE: A FOCUS ON EXTRACELLULAR VESICLES-MEDIATED COMMUNICATION

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Background: Multiple myeloma (MM) is an incurable cancer stemming from malignant plasma cells. MM is characterized by a strong tropism to the bone marrow (BM), where tumor cells accumulate and establish complex interactions with the normal stroma, which in turn promotes tumour survival, drug resistance and the development of bone disease. The Notch oncogenic pathway provides a key contribute to the ability of MM cells to shape the BM niche, affecting both BM cell biology and the intercellular BMstromal stromal microenvironment. Notch signaling in MM cells appears to be mediated by extracellular vesicles (EVs) that have been shown to be novel mediators in creating a supportive milieu for MM cells. Here we investigate the role of the activated Notch signaling in MM cells.

Aims: The aim of this work was to further elucidate the role played by the Notch pathway in the shaping of the BM microenvironment to provide a supportive niche for MM cells, with a focus on the contribution of EVs to the crosstalk between MM cells and the BM stromal cells.
Methods: We established two MM cell lines stably retaining the doxycycline-inducible pTRIPZ vector containing anti- Jagged1 and Jagged2 shRNAs and a BM mesenchymal stromal cell (BMSC) line expressing shRNAs for Notch1 and Notch2. EVs were isolated by ultracentrifugation and used for functional assays and molecular analysis. qPCR was performed using SYBR Green. Apoptosis analysis was performed by flow cytometry; evaluation of protein expression was performed by western blot analysis.

Results: We present evidences that EVs play a crucial role in the dysregulated interactions of MM cells with the BM microenvironment and that Notch regulates their release. Indeed, BMSCs knockdown for Notch1/2 results in a decrease in EVs release and reduce their ability to induce Bortezomib resistance in MM cells and to stimulate their migration. On the other side, MM-derived EVs are able to increase the production of pro-tumor factors by BMSCs (i.e. IL-6, TNFα), promoting their ability to boost tumor growth; interestingly, this effect is lost when EVs are isolated from MM cells where the Notch pathway was inhibited. Finally, EVs released by co-cultures of BMSCs and MM cells where the Notch pathway is blocked display a reduced ability to increase osteoclastogenesis compared to EVs from the control culture. These results are particularly relevant due to the crucial role played by bone disease in MM progression.

Summary/Conclusions: These new insights in the pathophysiology of the de-arranged BM niche represent the rationale for a Notch-directed therapy aiming to uncouple the crosstalk of MM with the surrounding microenvironment by inhibiting Notch signaling.

Background: Multiple myeloma (MM) remains largely incurable despite improvements in clinical outcomes following the approval of immunomodulatory drugs (IMiDs) and proteasome inhibitors (PIs) (Rajkumar et al 2010). Previous findings on the role of serum free light chain (sFLC) in the prediction of clinical outcome of therapy (DoT) with PIs and IMiDs (5 and 9 mo, respectively: Palumbo et al 2016) vs BMSCs clinical trials (Stewart et al 2014). Understanding real-world use of therapies for relapsed/refractory (RR) MM is important to determine their position in the treatment paradigm.

Aims: In this subsequent PREAMBLE analysis, treatment patterns in patients (pts) with RRM receiving bortezomib (bort) and carfizomib (carf) were evaluated to better understand the use of PIs in routine clinical practice.

Methods: PREAMBLE (NCT01838512) is an ongoing, observational, international cohort study exploring real-world treatment patterns and outcomes in pts with MM. Eligible pts were aged ≥18 yrs with diagnosis of RRMM and no prior treatment and were evaluated at the baseline media evaluation of therapy (DoT) with PIs and IMiDs (5 and 9 mo, respectively: Palumbo et al 2016) vs clinical trials (Stewart et al 2014). Understanding real-world use of therapies for relapsed/refractory (RR) MM is important to determine their position in the treatment paradigm.

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variable. Understanding the prognosis for a particular patient can help when selecting the intensity of treatment to be used and the frequency of reviews. The quantification of heavy/light chains pairs by the immunoassay Helyveil (HLC) allows us a precise measurement of monoclonal and non-monocononal immunoglobulins of the same isotype.

Aims: The aim of the study is to evaluate i) the impact of the “HLC ratio” defined as monoclonal immunoglobulin over isotype matched non-monoclonal immunoglobulin (involved/uninvolved HLC ratio or i/u HLC ratio), ii) the suppression on non-monoclonal pair denominated “HLC-matched pair suppression” and iii) the effect of “systemic immunoparesis” at diagnosis and at +100 days after autologous stem cell transplant (ASCT).

Methods: 85 patients (50 Male:35 Female) with a median age of 70 years (56-78) were followed (35 IgGK, 18 IgGL, 17 IgAK and 15 IgAL). The median follow-up of the patients was 19 (5-30) months. Sixteen patients (18%) presented ISS stage I, 15 (28%) with stage II and 54 (64%) with stage III disease. Thirty patients that reached ASCT were evaluated at +100 days after ASCT. Immunoglobulin heavy/light chains pairs (HLC) were assessed by Helyveil assays (The Binding Site). Clinical variables were evaluated for their impact on patient’s outcome. Overall survival (OS) and progression-free survival (PFS) were evaluated by Kaplan-Meier method and Cox regression. Statistical analysis was made with Prism 6.0.

Results: The median OS of the 85 patients was 54% and 26 patients deceased during the study due to MM. The median value of i/u HLC ratio was 80 (31.5-319.71). At diagnosis, a i/u HLC ratio>80 was significantly associated with worse OS (46 vs 61%, p=0,005) and shorter PFS (23 vs 42%, p=0,006). Severe HLC-matched pair suppression (i.e. more than 50% below the lower reference range) was identified in 68% of the newly diagnosed patients and was associated with significantly shorter OS (35% vs 81%, p=0,004) and PFS (21% vs 50%, p=0,013). Severe (>50%) systemic immunoparesis of non-monoclonal immunoglobulins was identified in 64% of the patients at diagnosis and was also significantly associated with shorter OS (32% vs 81%, p=0,030) but not with shorter PFS (26% vs 44%, p=0,306). The evaluation of other clinical variables on patient’s outcome are shown in table (see Table). In multivariate analysis, severe HLC-matched pair suppression and albumin were found as independent risk factors for OS whereas creatinine and i/u HLC ratio >80 were found as independent risk factors for PFS. In the post-ASCT evaluation of the patients after ASCT, severe HLC-matched pair suppression reflects the persistence of clonal cells that is not associated with severe systemic immunoparesis.

Summary/Conclusions: Severe HLC-matched pair suppression and i/u HLC>80 are associated with worse OS and shorter PFS in MM patients suggesting a potential use of these parameters as prognostic biomarkers in newly diagnosed patients. Severe HLC-matched pair suppression is an independent risk factor for OS whereas i/u HLC>80 is independently associated with shorter PFS. In patients after ASCT, severe HLC-matched pair suppression reflects the persistence of clonal cells that is not associated with severe systemic immunoparesis.

E1272

SURVIVAL STRATIFICATION OF PATIENTS WITH MULTIPLE MYELOMA (MM) AFTER FIRST RELAPSE: SENSITIVITY ANALYSES OF A NOVEL RISK STRATIFICATION ALGORITHM (RSA)

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Background: Established risk stratification tools in MM, such as the International Staging System (ISS) and the revised ISS, have improved overall survival (OS) estimates by combining the strongest known predictors of survival at diagnosis. There remains, however, a need for tools that use additional data available at relapse to improve risk stratification. We previously used real-world data from the Czech Registry of Monoclonal Gammopathies (RMG) to develop a RSA for estimating risk of death in patients with MM starting second line (2L) treatment. A multiple Cox regression model identified predictors of OS at 2L (Table); hazard ratios (HRs) for each predictor were multiplied to obtain an overall score for each patient. A K-adaptive partitioning for survival (KAPS) algorithm stratified patients into risk groups based on these scores.

Aims: To investigate how our RSA is affected by: 1) removing cytogenetic risk factors for OS whereas creatinine and i/u HLC ratio >80 were found as independent risk factors for PFS. In the post-ASCT evaluation of the patients (n=30), normalization of HLC ratio was observed in 22 patients (73%). An altered HLC ratio was significantly associated with shorter PFS after ASCT (25% vs 70%, HR: 3.42, 95% CI 1.12-11.97, p=0.039) and with a trend towards a worse OS (p=0.072). Severe HLC-matched pair suppression was found in 12 patients (40%) and was significantly associated with worse OS (32% vs 81%, p=0.030) but not with shorter PFS (26% vs 44%, p=0.306). On the other hand, the severe systemic immunoparesis observed in 17 patients (57%) was not associated with OS (p=0.644) and PFS (p=0.750).

Table 1.

Table 1.

Summary/Conclusions: Severe HLC-matched pair suppression and i/u HLC>80 are associated with worse OS and shorter PFS in MM patients suggesting a potential use of these parameters as prognostic biomarkers in newly diagnosed patients. Severe HLC-matched pair suppression is an independent risk factor for OS whereas i/u HLC>80 is independently associated with shorter PFS. In patients after ASCT, severe HLC-matched pair suppression reflects the persistence of clonal cells that is not associated with severe systemic immunoparesis.
Results: Results are shown in the Table. The model without CAs had similar HRs and predictors to the original; however, lactate dehydrogenase level at diagnosis was not identified as a predictor. Kaplan–Meier OS analysis showed separation between groups (median OS for the lowest [group 1] to the highest [group 4] risk group: 57.2, 29.4, 14.9 and 4.9 months), but the separation was weaker than when CAs were included in the model (median OS: 57.2, 28.8, 13.4 and 4.7 months). Despite 81% of patients in the RMG having no CA data, ‘missing’ CA was treated as a separate level in the original model. The fit of the model (measured using Akaike’s information criterion; Table) without CAs was worse than the original, reducing the accuracy of survival predictions. Adding 2L treatment as a predictor did not affect the model fit, indicating that OS predictions were not improved. KAPS analysis showed that a model with three groups for stratifying patients by risk of death was less effective than one with 4 or 5 risk groups. With group 1 as the reference, the HRs for OS were 2.4 and 8.1 for groups 2 and 3 in the three-group model (all p<0.001), 2.1, 4.2 and 11.1 in groups 2-4 in the four-group model (all p<0.001) and 1.8, 2.8, 4.9 and 10.5 for groups 2-5 in the five-group model (all p<0.001). Using five risk groups was considered less practical in a clinical setting than the four-group model, which provides a clearer difference in risk across groups.

Summary/Conclusions: These analyses indicate that our RSA incorporating data from diagnosis and relapse can identify patient groups with profoundly different survival expectations, regardless of 2L treatment type. CAs at diagnosis is a known OS predictor and, as expected, improves the strength of predictions. The practicalities of measuring CAs should be considered, but these data suggest that physicians should be encouraged to assess CAs at diagnosis; CAs at relapse may also be informative. Further validation of this model is required using other real-world and clinical trial data.

E1273
REAL-WORLD DATA ON MULTIPLE MYELOMA: A PROSPECTIVE NATIONAL REGISTRY IN URUGUAY ON 224 NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS FROM 2012-2015
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Background: The Uruguayan National Myeloma Registry is the first observational prospective Uruguayan registry designed to document clinical characteristics of newly diagnosed multiple myeloma (MM), treatment and outcomes in a real-world setting. It collects detailed data on MM patients diagnosed at all institutions from 2012, nationwide. Analysis of this non-selected data will allow us to plan strategies to improve our local approach to this disease, reducing problems derived from extrapolating information from other realities.

Aims: To document current strategies of clinical characteristics at diagnosis, management, outcomes and treatment adverse effects of non-selected newly diagnosed MM patients in a recent period.

Methods: This registry includes all MM diagnosed from January 2012 in all institutions, nationwide. Smoldering MM are not included. We present the analysis of the first 3 years of data collection. Information was obtained from medical records. The database includes clinical and laboratory characteristics, treatment, disease-related and treatment-related adverse events, response, progression free survival, overall survival and cause of death. Survival is obtained from the Uruguayan Ministry of Health database.

Results: With a 71% institutional coverage, 224 patients were included. Median age at diagnosis was 66 years (range 33-94 years), 54.5% were male; 10% were younger than 50 years and 34.5%, older than 70 years. Distribution according Ig subtype was: IgG 50.4%, IgA 23.3%, Light chains 18.7%, nonsecretor 2.2% and IgM <1%. Most patients had advanced disease: 79.6% Durie-Salmon stage ill (176/221), 48.6% ISS (86/177). Anemia (hemoglobin <10 g/dl) was present in 56%, osteolytic lesions in 69%, renal impairment (creatinine>2mg/dl) in 29.5% and hypercalcemia in 10%. Cytogenetics was evaluated in 150 patients; high risk features were detected in 6.3% by conventional cytogenetics and 19% by fluorescence in situ hybridization. First-line treatment included at least one of the new drugs (Thalidomide, Bortezomib or Lenalidomide) in 92% of patients ≤70 years and in 50% of >70 years. First-line response was available in 73%. Overall response rate (≥PR) was 82.3%, VGPR= 23.2% and CR=15.2%; 9.8% patients achieved stable disease and 7.9% were refractory. (Fig. 1). Comorbidities and treatment-related toxicities were observed in 43.6% (47% in ≥70 years) and 41%. Most common adverse events were recurrent infections (28%), neuropathy (17%), thromboembolic events (5.4%) and grade 3-4 cytopenias (5%). Sixty out of 146 potential candidates have been transplanted as first line consolidation at the time of this analysis. After a median follow-up of 30 months, overall survival was 62.8% (median NR in ≤70 years and 32 months in >70 years) and median progression free survival (PFS) was 17 months.

Summary/Conclusions: This first national registry provides a thorough insight into the characteristics of MM patients in our country. With a high institutional coverage, we show MM characteristics at diagnosis are similar to other real-life reports. (1) MM is detected in advanced stage with a high percentage of renal impairment. Diagnosis is performed according to international recommendations. First-line treatment is defined by local policies which restrict Bortezomib to high-risk cytogenetic features and/or renal impairment and do not provide Lenalidomide. Reasons for 59% potential candidates not receiving ASCT should be addressed in future research. This analysis provides relevant real-life information to plan strategies to improve MM management and perform high quality population-based research on the field.

Reference

E1274
REPRESENTATION OF MINORITIES, THE ELDERLY AND WOMEN IN MULTIPLE MYELOMA CLINICAL TRIALS
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Background: Multiple myeloma (MM) accounts for approximately 1% of all cancers and 10% of hematologic malignancies in the United States (US). MM occurs in all races but the incidence in African Americans is two to three times higher than in non-Hispanic whites. Many clinical trials (CT) lack appropriate representation of specific patient populations, limiting the generalizability of the evidence obtained.

Aims: Determine the representation of ethnic minorities, the elderly and women in MM CT.

Methods: Enrollment data from all therapeutic trials reported as completed in clinicaltrial.gov from 2000 to 2016 were analyzed. CT including other hematologic malignancies and with recruitment outside of the US were excluded. Enrollment fraction (EF) was defined as the number of enrollees divided by the 2013 Surveillance, Epidemiology, and End Results (SEER) database MM complete prevalence. Chi-square test was used to estimate differences in categorical data.

Results: Out of 177 MM CT, 78 (44%) reported ethnicity with a total of 12,055 enrollees. Out of those 78 CT, 52 (67%) were phase II, 15 (19%) phase III and 11 (14%) phase I. Most of the results were published from 2012 to 2016 (74%). Distribution by race, gender, age and comparison with the SEER MM prevalence data are described on Table 1. Forty-six (59%) trials were sponsored by industry, 7 (9%) by NCI and 25 (32%) were investigator initiated. Participation in CT varied significantly across ethnic groups, non-Hispanic Whites (NHW) were more likely to be enrolled in CT (EF of 0.23) than African Americans (AA) (EF of 0.08, p<0.0001) and Hispanics (His) (EF of 0.05, p<0.0001). Males had

Figure 1.
a higher recruitment rate than females (58% vs 42%), but this could be explained by the higher incidence of MM in this subgroup. Enrollee's median age was 62 years. Younger pts (<65 years) were more likely to be enrolled in CT than the elderly (66% vs 34%, p<0.0001). Industry sponsored trials were less likely to recruit AA compared with investigator initiated trials (7.6% vs 12%, p<0.0001).

Table 1.

<table>
<thead>
<tr>
<th>Race/Ethnicity</th>
<th>Patients Trained</th>
<th>% of Total</th>
<th>2013 MM Prevalence %</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>10,139</td>
<td>16.5%</td>
<td>20%</td>
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<tr>
<td>NA</td>
<td>371</td>
<td>5.9%</td>
<td>5.7%</td>
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<tr>
<td>Native American</td>
<td>150</td>
<td>2.4%</td>
<td>2.3%</td>
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<tr>
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<td>5.3%</td>
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</tr>
<tr>
<td>Native</td>
<td>2,271</td>
<td>3.6%</td>
<td>3.6%</td>
</tr>
<tr>
<td>Other Race</td>
<td>615</td>
<td>1.0%</td>
<td>1.0%</td>
</tr>
</tbody>
</table>

Summary/Conclusions: Despite the higher incidence of MM in African Americans and the elderly, the former only represented 8.6% of the study participants and 66% of these were less than 65 years of age, perhaps lacking data in the tolerability of these new agents in our aging MM population. We also observed industry studies were less likely to recruit AA patients. Future trials should take extra measures to recruit participants that adequately represent the United States MM population.

E1275

EVALUATION OF TREATMENT EFFICACY IN MULTIPLE MYELOMA AND ITS INFLUENCE ON PHYSICAL AND ROLE FUNCTIONING B. Sidi Mohamed El Amine,1*, H. Asma1, O. Fouza1, S. A. Najet1, Z. Zahi1 1Hematology department, University hospital of Sidi Bel Abbès, Sidi Bel Abbès, Algeria

Background: Peripheral neuropathy (PN) is a major dose limiting and potentially disabling adverse event of commonly therapeutic drugs used in the management of multiple myeloma (MM), including the immunomodulatory drugs (IMIDS, Thalidomide and Lenalidomide), and the proteasome inhibitor (Bortezomib).

Aims: The aims of this study were to (1) perform a psychometric evaluation of PN and (2) examine the prevalence of this complication and its influence on physical and role functioning of MM patients.

Methods: The FACT/GOG-Neurotoxicity (Ntx) subscale for assessing treatment induced PN was evaluated. The 11-item of this questionnaire was administered to patients with MM treated with IMIDS and/or Bortezomib. The subscale was evaluated in 32 patients for internal reliability, construct validity, criteria validity, and compared with other adjuvant agents (CTCAE version 3). Spearman rank correlation was calculated to determine the impact of PN on functional, physical and role functioning of MM patients, assessed by EORTC quality of life scale (EORTC QLG-C30). A Cronbach coefficient ≥ 0.8 is good. Spearman rank correlation is significant if p<0.05 or r >0,5.

Results: Cronbach alpha coefficient for internal consistency of FACT/GOG-Ntx subscale was 0, 92, and its correlation with the full CTCAE scale as follows: P=0,0001. All the 11 items exhibited high correlations with the NTX subscale score (r= 0, 65- 0, 79), and the Construct validity of NTX was good. According to FACT/GOG-NTX and NC-CTCAE, 24 (75%) patients presented PN secondary to IMID or Bortezomib. The PN was severe in 14 (43, 7%) patients, especially those who received Bortezomib associated with IMIDS (71, 4%). PN did not influence the achievement of a very good response of MM to therapy neither a complete remission (P=0,6), but patients with high scores of NTX subscale have reduced functional activities, especially physical and role functioning (P<0.0005, r=0,0001 respectively).

Summary/Conclusions: The 11-item FACT/GOG-Ntx subscale reliably and validly assesses Bortezomib/IMIDS induced PN. This complication is frequent and can alter the functional abilities of MM patients.

E1277

ANALYSIS OF THE CONNECT MM REGISTRY: TREATMENT OUTCOMES AND HEALTHCARE RESOURCE UTILIZATION IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA WHO RECEIVED LENALIDOMIDE MAINTENANCE OR NO MAINTENANCE R.M. Rifkin1,*, S. Jagannath2, B.G. Durie3, J.J. Shah4, M. Narang5, H.R. Terebelo6, C.J. Gasparetto7, K. Toomey8, J.W. Hardin9, L. Wagner10, K. Parikh11, S. Abouzaid11, S. Sriravasan11, A. Kitait11, F. Zafar11, R. Abonour12 1US Oncology Research, Rocky Mountain Cancer Centers, Denver, 2Mount Sinai Hospital, New York, 3Cedars-Sinai Samuel Oschin Cancer Center, Los Angeles, 4MD Anderson Cancer Center, Houston, 5US Oncology Research, Maryland Oncology Hematology, Columbia, 6Providence Cancer Institute, Southfield, 7Duke University Medical Center, Durham, 8Steeplechase Cancer Center, Somerville, 9University of South Carolina, Columbia, 10Wake Forest University School of Medicine, Winston-Salem, 11Celgene Corporation, Summit, 12Indiana University Simon Cancer Center, Indianapolis, United States

Background: Maintenance therapy post autologous stem cell transplant (ASCT) has been shown to improve clinical outcomes, including time to progression, progression-free survival (PFS), and overall survival (OS) in patients with newly diagnosed multiple myeloma (NDMM) (Sonneveld, N Engl J Med, 2012; McCarthy, N Engl J Med, 2012; Attal, N Engl J Med, 2012; Palumbo, N Engl J Med, 2014; Attal, ASCO, 2016). However, the effect of continued treatment on healthcare resource utilization (HRU) is mostly unknown. Connect MM is a largely community-based, US prospective observational cohort study designed to characterize diagnosis, treatment patterns, and outcomes in patients with NDMM in clinical practice.

Methods: Adult patients with NDMM were eligible for enrollment in the registry within 60 days of diagnosis. patients who completed induction and single ASCT without subsequent consolidation and received lenalidomide (LEN)-only or no maintenance were included in the analysis. HRU (hospitalization rates and length of stay, surgery/procedures, concomitant medications including growth factor, bisphosphonate, and neurophatic pain medication) was assessed from 100 days post-ASCT to the end of years 1 and 2. Data cutoff was Jan 7, 2016 and the median follow-up was 39.3 months.

Results: A total of 1493 patients with NDMM were enrolled in Cohort 1 from Sep 2009 to Dec 2011; 421 patients met the analysis criteria stipulated above. Within the cohort, 467 (32.2%) did not receive maintenance therapy. 256 received any type of maintenance therapy. Of those receiving maintenance, 180 (70%) were treated with LEN-only maintenance. The median age was 60 yr (range, 24-78); 60% were men, and 86% were white. Baseline patient characteristics except serum
creatinine, calculated International Staging System stage, history of monoclonal gammopathy of unknown significance, presence of del(17p), and induction regimen were similar across groups. LEN-only maintenance significantly extended PFS compared to no maintenance (median 54.5 months vs 30.8 months; hazard ratio [HR]=0.98 [95% CI: 0.43, 0.79]; P< 0.0005; Table). OS was also significantly improved with LEN-only vs no maintenance (HR=0.45 [95% CI: 0.29, 0.73]; P< 0.001). HRU results are detailed in the Table. The rate of hospitalization/100 person-years (PY) was similar across groups (P=not significant [NS], all comparisons) at the end of years 1 and 2. The median duration of hospitalization was numerically longer for patients who received no maintenance. Procedures/surgeries and concomitant medication use were similar across both groups at the end of years 1 and 2.

Table 1.

Summary/Conclusions: For patients with NDMM, LEN-only maintenance significantly improved PFS and OS vs no maintenance with no apparent impact on HRU.

E1278

SERUM-FREE LIGHT-CHAINS (SFLC) INSTEAD OF URINE PROTEIN ELECTROPHORESIS (UPEP) FOR MONITORING LIGHT-CHAIN MULTIPLE MYELOMA (LCMM)


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Background: Response and follow-up criteria in multiple myeloma (MM) are still based on the protein electrophoretic (PEP) quantification of the monoclonal protein (MP) in serum (s) and/or urine (u). Monitoring MP by urine (u) PEP has a very low sensitivity for evaluating variations of small amounts of MP. Since 2001, serum free light-chain assays (sFLC) are available, with demonstrated clinical utility. Dejoe et al. have recently reported the usefulness of sFLC for evaluating response in LCMM patients.

Aims: In this work, we try to validate the use of sFLC assay in the context of GEM/PETHEMA clinical trials in order to evaluate the responses and its advantages in comparison to standard quantification of MP by PEP in serum (s) and urine (u) studies in the usual clinical praxis.

Methods: We included 169 patients with Bence Jones (BJ) MM with measurable urine disease who have been treated according to GEM/PETHEMA clinical trials (GEM05menos65, GEM05MAS65, GEM2010MAS65 and GEM2012 menos65). Serum FLC assays (Freelite®, The Binding Site, Birmingham, UK) and proteinuria were performed on an automated nephelometer (BNII, Dade Behring / Siemens, Marburg, Germany). The electrophoretic study of the monoclonal component (CM) was performed by capillary electrophoresis (V8, Helena Biosciences Europe), and immunofluorescence was performed for the Ig, γ, κ and λ chains (SAS-3 and SAS-4, Helena Biosciences Europe).

Results: From a total of 169 patients with BJ, we included 76 (46%) patients with BJ and monoclonal kappa / 76 Bence Jones Lambda), 146 (86%) had FLC data at diagnosis, with 139/146 (95%) evaluable by FLCs [involved sFLC ≥100]. In addition, 68 of the 169 patients also had detectable MP in serum and 7 of the 169 had non-evaluable MP in urine (MP <0.200 g/24h). We studied the correlation of both techniques’ MP quantification results (uPEP vs sFLC) and we observed a low correlation (Pearson’s r=0.293, p=0.003), that should be partly explained by the low profitability and subjectivity of the electrophoresis technique for quantifying para-protein in urine. [Figure 1A]. The concordance between the classification of the response by uPEP / immunofixation (IF) and by FLCs (Kappa Index=0.425 [p<0.0001]) was in the range of 35/98 (36%) patients after treatment, associated to a lower risk of progression (normal vs abnormal sFLC: PFS 60 vs 39 months, p=0.038) but without impact in overall survival in our series. We also observed that an absolute value of sFLC greater than 50mg/L after treatment was associated with an increased risk of progression, regardless of the response achieved (PFS 60 vs 28 months, p<0.0001). [Figure 1B].

Summary/Conclusions: There is an acceptable agreement between both methods for response evaluation. The SFLC assays provide a greater sensitivity than the urine protein electrophoresis for monitoring low levels of disease in certain cases with measurable disease at diagnosis (sFLC ≥100) being useful for its follow-up, and also provide prognostic value as a predictor of progression.

E1279

TOPSPIN: A NOVEL ALGORITHM TO PREDICT TREATMENT SPECIFIC SURVIVAL IN CANCER

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Background: In recent years many novel treatments have been introduced for Multiple Myeloma (MM), leading to an improved survival. However, this has also led to the need for different treatment combinations and without a clear indication which patient will benefit most from which treatment. It is increasingly recognized that genetic heterogeneity between tumors influences treatment response. Patient outcomes may be improved by selecting the right treatment for the right patient at the moment of diagnosis. This requires the discovery of predictive markers, for example gene expression signatures, that can aid in this treatment decision. Here we present TOPSPIN (Treatment Outcome Prediction using Similarity between PatIeNts), a novel algorithm to discover such markers from tumor gene expression data. We use it to identify patients more likely to benefit from bortezomib.

Aims: This algorithm aims to develop a classifier that identifies a subset of patients that will benefit more from a treatment of interest than similar patients who receive a different treatment.

Methods: TOPSPIN aims to predict whether a patient will benefit (class 1) or will not benefit (class 0) from a certain treatment of interest based on the gene expression profile of the patient. This algorithm relies on the idea that genetically similar patients who received a different treatment should have a large difference in survival, given that genetic similarity is defined in a manner that is relevant to treatment response. This principle is used to identify prototype patients: patients who received the treatment of interest and have a larger than 0.581 gene sets based on Gene Ontology (GO) annotation. These prototype patients are used to define a classifier: new samples who exhibit a similar gene expression profile to the prototypes are also expected to benefit more from the treatment of interest. Here we use TOPSPIN to predict which patients will benefit from the proteasome inhibitor bortezomib. We combine tumor gene expression data from the Total Therapy 2, Total Therapy 3 and HOVON-65/GMMG–HD4 phase III clinical trials into one dataset comprising 910 patients, split into a bortezomib arm (n=407) and a non-bortezomib arm (n=503). Progression free survival is used as outcome measure. This dataset was split in a training set (n=606) and a test set (n=304). The test set is not used at any point in the training procedure and is only used for independent validation.
Results: We successfully identify gene sets that enable us to predict which patients will benefit most from bortezomib. The top 8 performing GO sets based on Hazard Ratio (HR) were combined to achieve the final classification. In the training set 28.4% of patients are classified a class 1, resulting in an HR of 0.13 (p=7.1*10-11) between the two treatment arms. More importantly, in an independent test set an HR of 0.47 (p=0.03) was found, as shown in Figure 1.

Figure 1. A Kaplan Meier of training set classification, showing a large survival benefit for patients receiving bortezomib in class 1 (red lines) but not in class 0 (blue lines). A. Training B. Test

Summary/Conclusions: TOPSPIN is successful in predicting bortezomb specific survival in independent data. TOPSPIN can be applied to any dataset with two treatment arms and a continuous outcome measure. In a disease like MM, where many different treatment are available, selecting the right treatment is critical and TOPSPIN can aid in this decision.

E1280

AMYLOIDOSIS RESEARCH CONSORTIUM CARDIAC AMYLOIDOSIS SURVEY: RESULTS FROM PATIENTS WITH AL AMYLOIDOSIS AND THEIR CAREGIVERS

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Background: Cardiac amyloidosis is a severe disease that can lead to cardiac dysfunction and death. Amyloid light chain (AL) amyloidosis, hereditary transthyretin (hATTR) amyloidosis, and wild-type transthyretin (wtTTR) amyloidosis may result in cardiac amyloidosis. AL amyloidosis is caused by an accumulation of misfolded light chain and often involves organs other than the heart (eg, kidneys, nervous system). Initial symptoms are often nonspecific (eg, weight loss, fatigue). Consequently, a diagnosis is frequently made only after the disease has become advanced. Previous patient-directed research found that despite patients being initially referred most often to cardiologists after the disease has become advanced. Previous patient-directed research found that despite patients being initially referred most often to cardiologists (as opposed to hematologists and nephrologists), cardiologists diagnosed the condition much less frequently than other specialists.

Aims: To understand delays, errors, and inconsistencies in the diagnostic pathway for patients with AL cardiac amyloidosis and validate using caregiver responses.

Methods: An online survey consisting of 36 questions (for patients) and 37 questions (for caregivers) was developed by the Amyloidosis Research Consortium (ARC) and distributed to the patient mailing lists of ARC, the Amyloidosis Foundation, and Amyloidosis Support Groups in January 2017. The survey was designed for patients with all forms of cardiac amyloidosis and their caregivers; however, the present analysis is limited to AL amyloidosis.

Results: In this subanalysis, 137 patients and 115 caregivers completed the survey. Most patient respondents were >55 years of age (n=111; 81.0%); of those, 16.1% (n=22) were >70 years of age. Composition of the population was 81.8% white/Caucasian (n=112), 2.2% Asian (n=3), 4.4% African American (n=6), 2.2% Latino (n=3), 5.1% other (n=7), and 3.6% unknown (n=6). Most patients had lived with their diagnosis for >1 year (17.5% [n=24] <1 year; 23.4% [n=32] 1-2 years; 29.2% [n=40] 3-5 years; 21.2% [n=29] 6-10 years; 8.8% [n=12] >11 years). A significant percentage of patients had multiorgan involvement (54.7% [n=75] kidney; 29.9% [n=41] nerve; 14.6% [n=20] liver; 43.8% [n=60] GI; 14.6% [n=20] skin; 22.2% [n=49] other site). Before diagnosis, 43.8% (n=64) of patients were incorrectly diagnosed with one or more other conditions, predominantly by cardiologists and general practitioners (Table 1). Furthermore, more than 75% of patients visited 3 or more different physicians before diagnosis. Nearly all misdiagnosed patients (83.3%; n=50/60) reported receiving treatment for their misdiagnosed condition. Both patients and caregivers reported most frequently by cardiologists and hematologists (Table 1). Caregivers echoed the multitude of distinct physicians visited before diagnosis (Table 1). Patients reported that biopsy of fat pad, kidney, or heart was the predominant diagnostic test performed (Table 1). Hospitalization was prevalent: 55.5% (n=76) patients reported amyloid-related cardiac hospitalization. Moreover, 31.3% (n=43) of patients reported the need for air travel for physician consultation.

Table 1.

Summary/Conclusions: This represents the first survey compiling both caregiver and patient experiences with AL amyloidosis. Alignment of caregiver with patient responses validates our patient-directed research. Patients with AL cardiac amyloidosis frequently receive misdiagnoses and sometimes receive incorrect treatment for the misdiagnosed condition. Disease awareness among all specialists is vital, especially among those to whom patients are initially referred due to the nature of their initial symptoms.

E1281

EFFECTICITY OF DARATUMUMAB-BASED REGIMENS IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA – A SYSTEMATIC LITERATURE REVIEW AND NETWORK META-ANALYSIS

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Background: Daratumumab is a new monoclonal antibody aimed to improve outcomes in relapsed or refractory multiple myeloma (RRMM), and has been investigated in combination with lenalidomide plus dexamethasone (DRD), and with bortezomib plus dexamethasone (DvD), in randomized controlled trials (RCTs), POLLUX and CASTOR, respectively. Although DRd and DvD have been compared against current standard of care (SOC), namely Rd and Vd, it is important to assess how daratumumab plus SOC compares with other routinely used treatment regimens and investigational regimens expecting regulatory approvals.

Aims: Therefore, the objective of this analysis is to compare DRd and DvD with other relevant treatment options via network meta-analysis (NMA) techniques.

Methods: A systematic literature review (SLR) based on searches of Medline, Embase, and the Cochrane Library was conducted to identify and then assess RCTs of treatments for RRMM. The specific studies of interest were those that included in a Bayesian NMA to allow for the indirect comparison.

Results: Data from RCTs identified by the SLR allowed formulation of two evidence networks. Network 1 included DRd and other immunomodulatory agent (IMiD)-containing regimens, and Network 2, contained DvD and other
immunomodulatory agent (IMiD)–free regimens. Analysis using a fixed-effects model showed that DRd compared with other IMiD-containing regimens in Network 1, and DvD compared with other IMiD-free regimens in Network 2 prolonged PFS and OS among patients with RRMM (see Table 1).

**Summary/Conclusions:** In the absence of prospective head-to-head trials, NMA provides potentially important information on comparative effectiveness of different treatments. This MAA suggests that the combinations of DRd and DvD may be more effective than PFS in patients with RRMM with similar trends found for OS when compared with other established and new regimens.

**E1282**

**TRENDS IN TREATMENT PATTERNS AND SEQUENCING IN PATIENTS WITH MULTIPLE MYELOMA DIAGNOSED 2011-2016 IN THE UNITED STATES USING AN ENHANCED ELECTRONIC HEALTH RECORDS DATABASE**

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**Background:** Over the past few years, the multiple myeloma (MM) treatment (Tx) landscape has changed considerably. Immunomodulating (IMiD) drugs and proteasome inhibitors (PI) have emerged as mainstays of MM Tx. However, the limitations and lag time of available administrative claims databases make it difficult to assess current real-world trends in the Tx of MM.

**Aims:** The study aimed to describe trends in demographics, Tx patterns, and sequencing for newly diagnosed MM (ndMM) patients (Pts) in the United States (US) using an enhanced Electronic Health Records (EHR) database.

**Methods:** A retrospective observational study of ndMM Pts was conducted utilizing EHR from a nationally-representative database (Flatiron Health). The Flatiron MM provider network comprises over 260 clinics throughout the US. Pts with an ICD-9 (203.0x) or ICD-10 (C90.xx) diagnosis of MM between 01/01/2011–12/31/2016 were randomly selected into the study. Pts were excluded if they did not have ≥2 documented clinical visits during the study period. Diagnosis of MM was confirmed through review of unstructured chart data. Index date was defined as the Pt’s date of diagnosis with MM. NdMM Pts were defined as those without a MM Tx more than 14 days prior to their first diagnosis date. Start of first-line (1L) therapy was defined as the 1st episode of an eligible systemic Tx given after or up to 14 days before the index date. Regimens were defined using the 1st eligible drug episode plus other eligible drugs given within 28 days of each other. A maximum gap of 90 days was allowed within a given line of therapy (LOT) and was considered concluded the day before the start date of the next LOT.

**Results:** For the 3367 ndMM Pts identified, mean(SD) age was 68.5(11) years at the time of diagnosis, 45.9% were female, 57.6% were white, 14.7% African American, and 11.1% other race. The most common immunoglobulin (Ig) classes at diagnosis were IgG (51.8%) and IgA (18.9%). Median follow-up time for ndMM Pts was 15.9 months. During the study period, 1611 received only 1 line (L), 1617 were treated with 2L, 325 with 3L, 252 with 4L+; while 442 (13%) received no Tx. Mean follow-up time for these groups was 471, 730, 928, 1132, and 610 days respectively. Among treated Pts, 205 (12.7%), 208 (28.2%), 109 (33.5%), and 98 (38.1%) received at least 1 stem cell transplant (SCT), respectively. Of Pts receiving 1L therapy, 984 (33.6%) received IMiD compound +PI, 712 (21.7%) received PI-only therapy, and 556 (17%) received IMiD compound-based therapy in 1L. The use of IMiD compound +PI in 1L increased during the study period for SCT and non-SCT Pts (NSCT) from 40.6% and 21.5% in 2011, to 66.7% and 46.8% in Pts diagnosed in 2016. In Pts who received a SCT (n=618), the most common 1L regimens were lenalidomide + bortezomib + dexamethasone (RVd; n=217, 43.9%), cyclophosphamide + bortezomib + d (CyBord; n=124, 20.1%), lenalidomide + dexamethasone (Rd; n=70, 11.3%), and bortezomib + dexamethasone (Vd; n=57, 9.2%). In NSCT Pts (n=2307), the most common 1L regimens were CyBord (13.2%), carfilzomib monotherapy (7.4%), pomalidomide + d (Vd; n=57, 9.2%). In NSCT Pts during the study period was 23%; and 20 patients (18%) died within 6 months from diagnosis. We found a significant association between HLC pair suppression and both the occurrence of bloodstream infections (OR: 6.10, 95% CI: 1.71-21.83; p=0.002) and early deaths (OR: 4.02, 95% CI: 1.10-14.66; p=0.03); by contrast SI had no significant association with either event (p=0.07 and p=0.3, respectively). Survival analyses demonstrated an association between bloodstream infections and shorter OS (50% vs 92%; HR: 7.43, 95% CI: 2.96-18.61, p<0.0001, Figure A). The risk of bloodstream infections was significantly higher among patients with HLC pair suppression compared to those without suppression (43% vs 7%, respectively; HR: 5.12, 95% CI: 1.54-17.07, p=0.003, Figure B). In line with this, patients with HLC pair suppression had shorter overall survival (OS) compared to those without (76% vs 93%; HR: 3.47, 95% CI: 1.02-11.83, p=0.03). By contrast we found no association between SI and risk of infection (p=0.08) or survival (p=0.4).

**Figure 1.**

**Summary/Conclusions:** HLC pair suppression provides information on immune status and associates with an increased risk of bloodstream infections and early deaths in newly diagnosed MM patients. Our findings highlight the importance of recognising this status at time of diagnosis, and suggest that HLC pair suppression may help guide clinical decisions about the need for adequate antimicrobial treatment during myeloma therapy.

**E1284**

**DARATUMUMAB SIGNIFICANTLY IMPROVED PROGRESSION-FREE SURVIVAL IN COMBINATION WITH LENALIDOMIDE AND DEXAMETHASONE IN PATIENTS WITH RELAPSED MULTIPLE MYELOMA**

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**Background:** Daratumumab is a human IgG1k monoclonal antibody which binds with high affinity to the CD38 molecule on the surface of multiple myeloma cells and induces rapid tumor cell death through multiple immune-mediated mechanisms and showed encouraging results alone and with lenalidomide and dexamethasone in a phase I-2 study involving patients with relapsed multiple myeloma.

**Aims:** The primary end point of the study was progression-free survival (PFS). We enrolled a total of 134 patients (74 males, 60 females; mean age 65.4±18.2 years) with multiple myeloma who had received at least three lines of therapy to receive lenalidomide with dexamethasone (68 patients, control group A) or in combination with daratumumab (66 patients, therapy group B).

**Summary/Conclusions:** Daratumumab is a human IgG1k monoclonal antibody which binds with high affinity to the CD38 molecule on the surface of multiple myeloma cells and induces rapid tumor cell death through multiple immune-mediated mechanisms and showed encouraging results alone and with lenalidomide and dexamethasone in a phase I-2 study involving patients with relapsed multiple myeloma.
Results: At a median follow-up of 9.8 months in a protocol-specified interim analysis, 67 patients had disease progression or death were observed (in 18 of 66 patients (27.2%) in the group B vs 28 of 68 (41.1%) in the control group (p<0.001)). A significantly higher rate of overall response was observed in the group B than in the group A (68.7% vs 62.9%, p<0.001), as was a higher rate of complete response or better (39.2% vs 16.1%, p<0.001). The most common adverse events during the treatment was myelotoxicity (neutropenia in 68.6% of the patients in the therapy group B vs 42.1% of those in the control group A), anemia (in 21.5% vs 13.6%) and thrombocytopenia (in 13.8% vs 8.7%).

Summary/Conclusions: In patients with relapsed multiple myeloma, the addition of daratumumab to lenalidomide and dexamethasone appeared active and resulted in significantly improved progression-free survival. However it was associated with a higher risk of myelotoxicity.

E1285
COMPARISON BETWEEN IMMUNOFIXATION NEGATIVITY AND NORMAL FREE LIGHT CHAIN RATIO WITH MULTICOLOUR FLOW CYTOMETRY FOR MRD ASSESSMENT IN PATIENTS WITH MULTIPLE MYELOMA WITH VGPR OR BETTER
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Background: Urine and serum Immunofixation electrophoresis (uIFE and sIFE, respectively) and free light chain assay (FLC) are widely accepted as standard tests for diagnosis and monitoring of multiple myeloma (MM). However, there is significant discordance between the electrophoretic method and FLC test for response assessment. Despite this discordance, previous studies did not address the differences in assessment of treatment response between the intact immunoglobulin MM (IIMM) and light chain only MM (LCMM)/oligosecretory MM (OSMM). uIFE results are poorly correlated with the serum FLC level, however, treatment response of LCMM has still been recommend to assess by 24-hour uIFE by International Myeloma Working Group guideline. However, MRD levels on uIFE negativity or normal FLC ratio (rFLC) in patients with various types of MM have not been studied.

Aims: To explore the relationship between uIFE, sIFE negativity and normal rFLC for MRD assessment in patients with IIMM and LCMM.

Methods: We initially selected 162 patients with MM (LCMM and OSMM, n = 41; IIMM, n=21) that received treatment at Kameda Medical Center, Kamogawa-shi, Japan and Kanazawa University Hospital, Kanazawa-shi, Japan between April 2008 and January 2016. Among them, 126 patients (LCMM/OSMM 40, IIMM 86), who achieve VGPR or better response, were selected on the basis of the availability of simultaneous serum and urine test, FLC data, and bone marrow MRD. To explore the relationship between uIFE and sIFE negativity and normal rFLC, MRD levels were compared by multi-colour flow-cytometry (MFC) in patients with LCMM/OSMM, and IIMM that obtained VGPR or better. MRD negativity was defined as MRD < 10-4. Complete response (CR) was divided into conventional CR (cCR, CR but MRD-positive) and MRD CR (CR and MRD-negative).

Results: One hundred forty-four complete IFE, FLC, and MFC data set of 126 patients (LCMM/OSMM 40, IIMM 86) with > 2 VGPRs were analysed. Normal FLC at VGPR, cCR and MRD- CR was 65.0%, 78.4% and 78.6% in IIMM, and 0%, 21.4% and 100%, respectively, in LCMM/OSMM. The percentages of sample at MRD levels of MRD >10-3, 10-3 ≤MRD >10-4, and 10-4 ≤MRD to the intact immunoglobulin MM (IIMM) and light chain only MM (LCMM) oligosecretory MM (OSMM) significantly differed. IIMM MRD at 12.5%, 50.0%, and 100% for negative uIFE, and 0%, 11.5% and 100% for normal rFLC, respectively. These figures in IIMM were 23.0%, 41.6%, 81.4% for negative sIFE, and 53.8%, 75.0% and 88.8% for normal rFLC, respectively. Positive/negative predictive value (PPV/NPV) of uIFE and rFLC for MRD in LCMM/OSMM was 100%/54.8% and 100%/85.0%, respectively. Positive/negative predictive value (PPV/NPV) of negative sIFE and normal rFLC are still useful for response assessment in LCMM/OSMM as an alternative to 24-h uIFE, and both negative sIFE and normal rFLC are still useful for response assessment of residual clonal PCs in IIMM.

Summary/Conclusions: Our observations confirmed that FLC test has greater sensitivity than uIFE for detection of the monoclonal component, and that normalization of sFLC ratio is highly predictive of MRD negativity in patients with LCMM/OSMM. The proportion of negative sIFE samples increased with depth of MRD, but the FLC response did not appear to parallel with the depth of response in IIMM. We recommend that FLC test should be incorporated into evaluation of MRD assessment in LCMM/OSMM as an alternative to 24-h uIFE, and both negative sIFE and normal rFLC are still useful for response assessment of residual clonal PCs in IIMM.

E1286
DARATUMUMAB IS AN EFFECTIVE AND SAFE SALVAGE THERAPY IN RELAPSED/REFRACTORY PATIENTS WITH MULTIPLE MYELOMA AFTER ALLOGENEIC STEM CELL TRANSPLANTATION
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Background: Daratumumab is a human monoclonal antibody that targets CD38, a cell surface protein that is overexpressed on multiple myeloma cells. The drug became the first monoclonal antibody as single agent therapy approved by the FDA for the treatment of multiple myeloma. The role of allo-SCT in myeloma patients (pts) remains unclear; nevertheless, the registry study of EBMT suggests an increasing rate of allografts in Europe in last years. Despite the potentially curative potential of this approach, the increased relapse rate and low PFS remain a central clinical problem.

Aims: In this single center retrospective analysis, we report on our experience on the use of daratumumab in relapsed/refractory myeloma pts after allo-SCT.

Methods: A total of 16 pts (male, n=9) with median age of 66 years (39-72) relapsing after allo-SCTs that had been performed during a period 2008-2015 at the University of Hamburg and received daratumumab as single agent salvage therapy. Before allografting 9 pts received one and 7 pts 2 autologous, respectively. All but one pt received at least 1 salvage therapy line prior to the allo-SCT. The allografts were performed from unrelated donors (MUD, n=9; MMUD, n=4) or matched related donors (MRD, n=3). The median number of salvage lines post-transplant and prior to first daratumumab infusion was 3 (1-4). The salvage regimens included bortezomb, lenalidomide, pomalidomide and daratumumab. Daratumumab infusions were started at a median of 21 months (0-30) after relapse/progression.

Results: The median number of infusions was 13 (3-22). A total of 16 and 15 pts were available to safety and efficacy evaluation, respectively. The safety was assessed according to the Common Toxicity Criteria (CTC). A total of 20 adverse events were observed in 16 pts: dyspnea (CTC1, n=3; CTC2, n=1), bronchospasm (CTC2, n=2) shivering (CTC1, n=3), cough (CTC1, n=1; CTC2, n=1), musculoskeletal pain (CTC1, n=4), acute coronary syndrome (CTC3, n=1), skin rash (CTC2, n=1), pressure on ears (n=1). Two patients developed late onset infections (pneumonia and infection of urinal tract) followed by temporary therapy interruption. We observed a decrease of Tregs (CD4+CD25highFoxPD1high) flow num- ber from a median of 5.05% at start to 0.65% at day 21 after first daratumumab infusion in four pts. There were no cases of GvHD. The adverse events appeared in all pts after the first infusion, with improved tolerance of following infusions. There were no cases, where the therapy had to be stopped due to adverse events. Within a median follow-up of 32 months (1-45) from the relapse/progres- sion 12 of 16 pts remain alive. Two pts died due to progress of myeloma and another 2 pts died due to severe infection/sepsis. A total of 9 of 15 evaluable pts responded (60%, PR, n=7, vgPR, n=2). The responses (decrease of paraprotein and/or free light chains; reduction of extramedullary tumor in 2 pt) occurred at a median of 7 days (7-75) after the first administration of daratumumab. The median response duration is 4.5 mo (1.5-8). Six pts show ongoing responses. All responding 2 non-responding pts (stable disease) showed clinical improvement of constitutional symptoms.

Summary/Conclusions: Daratumumab demonstrated encouraging efficacy in relapsed refractory pts with myeloma after allo-SCT. The administrations of the drug in these heavily pre-treated pts were associated with good safety profile and development mostly non-severe adverse events mostly after the first infusion. Further studies on the use of daratumumab in post-transplant setting are warranted.
Background: Vascular endothelial growth factor (VEGF) is a potent angiogenic peptide with biologic effects that include regulation of extracellular matrix remodelling and inflammatory cytokine generation with an important role in the bone marrow microenvironment of multiple myeloma (MM). Angiogenesis is heightened in the bone marrow of MM patients in parallel with tumor progression. Myeloma and stromal cells secrete angiogenic factors that include VEGF. Previous studies showed significant heterogeneity in the expression of VEGF between plasma cells (PCs) from the same MM patient. However, no clear association with expression levels, phenotypic subtypes of PCs and prognosis was demonstrated.

Aims: The present study aimed to evaluate the expression levels of VEGF and VEGF receptor (VEGFR) on phenotypic subtypes of PCs in patients with monoclonal gammopathies and to explore its role as diagnostic and prognostic biomarkers.

Methods: We include 128 patients with monoclonal gammopathies, 60 patients with newly diagnosed symptomatic MM and 68 with monoclonal gammopathy of uncertain significance (MGUS) and also from 11 non-neoplastic controls (CN). Inclusion criteria were patients diagnosis levels of VEGF and VEGFR by flow cytometry in the two populations of bone marrow PCs, identified by gating CD138+CD19- (clonal PCs) and CD138+CD19+ (non-clonal PCs). The results are presented as percentage of PCs expressing VEGF/VEGFR and as expression levels of this antiangiogenic molecules expressed in mean intensity of fluorescence (MIF). The effects of these parameters on progression-free survival (PFS) and overall survival (OS) were analyzed with Kaplan-Meier method. For statistical analysis, software IBM SPSS Statistics v22 was used. ROC curves were performed to assess the VEGF and VEGFR accuracy as diagnostic and prognostic biomarkers.

Results: In our cohort of patients, median age was 70 (39-86) years, 52% were male. We found increased expression levels of VEGF in CD138+CD19- PCs from MM (80±7.5 MIF) compared toMGUS patients (61±7.6 MIF) (p=0.011), and also higher to the observed in CD138+CD19+ PCs (39.9±1.74 MIF) in both populations of patients (p<0.001 and p=0.02, respectively). No difference was found when comparing the expression levels of VEGF in CD138+ D19+ PCs from MM (39.9±1.74 MIF),mgUS patients (41.1±1.92 MIF) and controls (32.8±1.5 MIF). However, the percentage of CD138+CD19+ cells expressing VEGF was significantly higher inmgUS (39.44%) and in MM patients (48.74%,5%) compared to CN (13.5±5%,p=0.019 and p=0.003, respectively). The differential expression of VEGF showed thatMGUS patients with VEGFR levels higher than 23.5 MIF in CD138+CD19- PCs have higher probability to progress to MM [AUC 0.688 (95%CI 0.592-0.784), p=0.001, 90% specificity, 65% specificity, 65% PPV, 84% NPV]. In MM patients, we also found an association between increased VEGF expression levels in CD138+CD19- PCs (17.5%) and inferior OS (p=0.003) and OS (p=0.003), irrespective of first line therapy (bortezomib-based regimens for patients fit or alkylating-based treatments for unfit patients). Interestingly, we also observed an increased percentage of CD138+CD19+ PCs (21%) expressing VEGF in MM patients with a more favorable PFS (p=0.04) and OS (p=0.008).

Summary/Conclusions: The results of our investigation showed that CD138+CD19- and CD138+CD19+ PCs have differences in what concerns VEGF/VEGFR expression, not only in MM patients, but also inmgUS patients. The increased expression of VEGF in clonal PCs from MM compared toMGUS patients evidences the relevance of VEGF in myelomaogenesis. We also demonstrated a negative prognostic impact of an increased VEGF expression in CD138+CD19- PCs, highlighting the role of VEGF in the survival and maintenance of clonal PCs and as a predictor of outcome in MM progression. The association between the percentage of CD138+CD19+ PCs and survival supports the hypothesis that these cells may not be neutral players in the complex pathogenesis of MM. The results of our study should be further investigated in larger series of patients.

E1288
RACIAL DIFFERENCES OF FISH ABNORMALITIES IN MINORITIES WITH MULTIPLE MYELOMA: A SINGLE.CENTER EXPERIENCE
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Methods: IGH rearrangements (IGH r), t(4;14), t(11;14), and high risk: t(14;20), largest cohort of minorities to date.

Aims: To explore racial-based differences of FISH abnormalities using the largest cohort of minorities to date.

Methods: We included 128 patients with monoclonal gammopathies, 60 patients with newly diagnosed symptomatic MM and 65 with monoclonal gammopathy of uncertain significance (MGUS) and also from 11 non-neoplastic controls (CN). Chi-square was used for statistical analysis.

Results: Of the 128 patients included, 34 (26.7%) had FISH abnormalities, of which t(14;16), del13q, del 17p, 1q21. Chi-square was used for statistical analysis.

Table 1.

|          | W | M | E1289
POMALIDOMIDE ALONE OR IN COMBINATION WITH LOW DOSE DEXAMEThASONE AS MAINTENANCE INDUCTION WITH POMALIDOMIDE AND LOW DOSE DEXAMETHASONE IN RELAPSED AND REFRACTORY MYELOMA (ALLM MM14)
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Background: Whilst the addition of dexamethasone (DEX) to pomalidomide (POM) has been shown to improve clinical outcomes in patients with relapsed and refractory multiple myeloma, whether the addition of DEX to POM-LoDEX (Arm 2) or better to the standard of care (SD) or better to Cig salvage treatment for unfit patients. Interestingly, we also observed an increased percentage of CD138+CD19+ PCs (21%) expressing VEGF in MM patients with a more favorable PFS (p=0.04) and OS (p=0.008).

Summary/Conclusions: The results of our investigation showed that CD138+CD19- and CD138+CD19+ PCs have differences in what concerns VEGF/VEGFR expression, not only in MM patients, but also in MGUS patients. The increased expression of VEGF in clonal PCs from MM compared to MGUS patients evidences the relevance of VEGF in myelomaogenesis. The present study aimed to evaluate the expression levels of VEGF and VEGFR receptor (VEGFR) on phenotypic subtypes of PCs in patients with monoclonal gammopathies and to explore its role as diagnostic and prognostic biomarkers.

Methods: We include 128 patients with monoclonal gammopathies, 60 patients with newly diagnosed symptomatic MM and 65 with monoclonal gammopathy of uncertain significance (MGUS) and also from 11 non-neoplastic controls (CN). Chi-square was used for statistical analysis.
**Background:** Multiple myeloma is a heterogeneous disease that accounts for approximately 10% of all haematological malignancies. While European treatment guidelines exist for multiple myeloma, there is limited understanding about the characteristics of patients with multiple myeloma in Europe and how these characteristics vary by disease stage. Numerous patient and disease-related factors can have an impact on treatment choice. Data surrounding these factors would help to better characterise European patients and inform management and treatment practices in multiple myeloma.

**Aims:** The aim of the current study is to describe multiple myeloma patients from 5 European countries (France, Germany, Italy, Spain, and the UK) across the disease continuum.

**Methods:** Data were drawn from the Adelphi Real World Multiple Myeloma Disease-specific Programme (DSP), which was conducted across France, Germany, Italy, Spain, and the UK in Q1 2015. The Multiple Myeloma Group (MMG) is a real-world, cross-sectional survey that involves haematologists and haematologists who treated patients with multiple myeloma and was conceived to provide in-depth insight into the characteristics of patients with multiple myeloma treated in the real-world setting.

**Results:** A total of 262 physicians reported on 2,024 patients with multiple myeloma. Of these patients, 73.2% were receiving first-line treatment; the remaining 26.8% were receiving second-line treatment or later. The median age of multiple myeloma patients was 70 years; 58.4% were male, and most patients (88.5%) were white/Caucasian. Only 4.3% of patients had a family history of cancer. Patients had a mean height of 168.8 cm, a mean weight of 72.8 kg, and a mean body mass index of 25.5 kg/m^2^.

**Summary/Conclusions:** Results from this analysis provide valuable insight into myeloma patients in European countries. These findings can help to inform future treatment practices in Europe.
stable disease and one progressed during the 4th cycle of treatment. After ASCT the ORR was 84.4% (613.3%) patients achieved CR, 13 (28.9%) VGPR and 19 (42.2%) PR. Adverse events of grade 3 or 4 included mainly anemia (4 patients, 9%), neutropenia (3, 6.6%) and febrile neutropenia (one patient). After a median follow-up of 1.9 months (range: 1.0-24.9), 11 patients have progressed and 4 died (all had achieved less than VGPR post-ASCT). The PFS, TTP and OS rates at 12 months were 88.6%, 88.6% and 100%, respectively. Forty (89%) patients had adequate stem cell collection post-RAD induction (meanSD: 8.94±6.50 x1010/kg CD34+ cells). Patients at baseline had elevated levels of CTX, TRACP-5b, sRANKL/OPG, Dkk-1, Ang, VEGF, VEGF-A, bFGF and reduced levels of Angpt-1/Angpt-2, bALP and PINP compared to healthy subjects of similar age and gender (p<0.01 for all comparisons). RAD therapy resulted in a reduction of circulating CTX (p=0.03), TRACP-5b (p=0.01), Ang (p=0.02), VEGF (p=0.01) and bFGF (p<0.01). Moreover, RAD increased serum levels of bALP (p=0.036), PINP (p=0.028) and Ang-1/Ang-2 ratio (p=0.022). These alterations occurred irrespective of response, although patients who achieved at least VGPR tended to have more profound differences in the above parameters.

Summary/Conclusions: RAD resulted in successful induction for NDMM patients with an ORR of approximately 67% pre- and 84% post-ASCT. With a median follow-up of >1.5 years, the 12-month PFS rate and OS rates are high, as expected. RAD reduced bone resorption and increased bone formation; the latter has not been previously described with lenalidomide-based regimens. Furthermore, RAD reduced angiogenic cytokines and this supports the action of the regimen also through the disruption of the interactions between myeloma and stromal cells.

E1293

MULTIPLE MYELOMA IN THE REAL WORLD: HOW THERAPEUTIC LANDSCAPE HAS CHANGED IN THE LAST 15 YEARS

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Background: Therapeutic Multiple Myeloma (MM) scenario has completely changed in the last 15 years: conventional chemotherapy (CT) has been gradually abandoned and autologous stem cell transplantation (ASCT), proteasome inhibitors as Bortezomib (Bor) and immunomodulatory drugs as Thalidomide (Thal) have become the new actors in MM treatment (Tx).

Aims: aim was to outline how the management of MM patients (pts) had changed in the last 15 years reporting the experience of a single center.

Methods: Overall survival (OS) was measured from disease onset to death for any cause or last follow up. Progression free survival (PFS) was defined as the time from first-line to disease progression or last-follow-up. The effect of these parameters.

Table 1.

Results: We analyzed 584 MM pts diagnosed in our center from 2000 to 2015. Patients' characteristics are reported in Table1. Median number of therapy lines is 2 (1-9). Among pts ≤65 yrs, 242/371 (71.8%) received ASCT as 1st line tx. Patients >65 yrs were treated as follows: 16 (8.5%) received ASCT, 53 (28.2%) other therapies. As 2nd line tx our pts received: 27 ASCT (8.9%), 115 Bor-based tx (38.1%), 48 Len-based tx (16.5%), 53 CT (17.5%) and 59 other therapies (19.5%). As 3rd line tx 5 pts received ASCT (2.8%), 65 Bor-based tx (35.9%), 42 Len-based tx (23.2%), 39 CT (21.5%) and 30 other therapies (16.6%). The percentage of pts receiving a new drug in 1st line was 64% (338/525). This percentage was significantly different in pts treated before and after 2007 (42% vs 87%; p<0.001). Similar results were observed in 2nd line, 75% of pts treated before 2007 received a new drug and 90% after 2007 (p=0.002). Median PFS in pts >65 yrs was 1.7 vs 2.4 yrs (p<0.001); median PFS in pts ≤65 yrs receiving or not ASCT was 3.2 vs 1.9 yrs (p=0.001); of note, PFS was not different when considering pts undergoing to ASCT after a CT-based or a Bor-based induction (3 vs 2.5 yrs, p=0.2). Time to next treatment (TTNT) in pts receiving ASCT or not was 30.1 months (5-122.7) vs 10.3 months (0.7-70.5) (p<0.001) from 1st to 2nd line tx and 11.2 months (0.3-121.9) vs 6.3 months (141.6) from 2nd to 3rd line tx (p=0.026). The early mortality (within the first year) was 5.9% (31/525), in details only 1/258 of those eligible to ASCT (0.4%) and 30/257 of those not candidate to transplant (11.2%). When considering this last group before and after the 2007, we observed a significant higher incidence of early mortality in the first period [21 (17.2%) vs 9 (6.2%); p=0.006]. About new drugs toxicity: with Bor-based tx 30% of pts complained neurological, 20% gastrointestinal and 18.2% hematologic toxicity; with Len-based tx 36.4% infective events and 28.9% hematologic toxicity. Median OS in pts ≤65 yrs was 7 vs 4.8 yrs (p=0.001), of note considering pts ≤65 yrs treated before 2007 median OS was 5.5 vs 3.1 yrs (p=0.001) and after 2007 median OS was not reached vs 7.5 yrs (p=0.034).

Summary/Conclusions: Our real life data show how MM therapeutic scenario have changed during the last 15 yrs. The tremendous improvement observed in this study was mainly evident in older pts with a strong reduction of early mortality and median OS reaching, in the second time frame after year 2007, 7.5 yrs. For younger pts ASCT confirmed to be of great benefit in term of TTNT and PFS. Thus, considering the real advantage of new drugs a palliative approach is not anymore justified even in very old pts.

E1294

CUL4A EXPRESSION AS A POTENTIAL PROGNOSTIC marker IN MULTIPLE MYELOMA PATIENTS TREATED WITH IMMUNOMODULATORY DRUGS

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Background: Despite the clinical effectiveness of immunomodulatory drugs (IMiDs) in multiple myeloma (MM), neither their mechanisms of action nor the biomarkers that could identify patients who would benefit from IMiD therapy are yet known. While the identification of the IMiDs action via cereblon (CRBN), Ikaros (IKZF1) and Aiolos (IKZF3) was a milestone, the role of other pathways including CRBN and E3 ubiquitin ligase complex proteins (CUL4A, DDB1, Roc1) are not fully understood so far.

Aims: The aim of this study was to: 1) evaluate CUL4A, IKZF1, IKZF3, MUM1 and IRF4 expression in bone marrow trephine biopsies obtained from multiple myeloma patients before treatment with thalidomide, 2) analyze the associations between the expression of these proteins and clinical characteristics and outcomes.

Methods: IHC staining for CUL4A, IKZF1, IKZF3, MUM1 and IRF4 expression was performed in trephine biopsies obtained from 25 patients with multiple myeloma before the treatment initiation. The patients (20 females, 5 males, median age 68 years) were treated with thalidomide based regimens as a first line treatment. The patterns of proteins' expression were scored independently by two hematopathologists on a semi-quantitative scale and the cutoff was defined as ≥ 30% positive cells. Associations between studied proteins' expression and clinical parameters were assessed using Fisher’s Exact Test for categorical variables and Mann-Whitney-Wilcoxon Test for continuous variables. Survival (PFS and OS) were estimated using the Kaplan-Meier method and censored using the log-rank test.

Results: Prior to treatment with thalidomide, 13 patients (52%) showed high expression (≥30%) of CUL4A protein. No associations between expression of CUL4A and other proteins were seen. Patients with high CUL4A expression more often presented low disease stage according to Durie-Salmon classification (P=0.02), beta-2-microglobulin level within normal ranges (P=0.07) and higher median platelet count (P=0.003) compared to patients with low CUL4A expression. Moreover, patients with high CUL4A expression before treatment showed longer PFS compared to those with low CUL4A expression (P= 0.03).

Additionally, a significant association between high Aiolos expression and high E3 ubiquitin ligase complex proteins expression was observed. In CD138+ cells with bone marrow was observed a higher median platelet count (P=0.01) compared to low Aiolos expression, however no other associations with clinical course of MM patients were seen. No associations between IKZF1, IKZF3, IRF4, MYC expression and patients' characteristics or outcome were revealed.
E1295

MAINTENANCE THERAPY WITH BORTEZOIMB IN PATIENTS WITH MULTIPLE MYELOMA (MM) AFTER ASCT AND MINIMAL RESIDUAL DISEASE (MRD)

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Background: MRD-negativity status in patients with MM after autologous stem cell transplantation (ASCT) directly correlates with higher Relapse-Free Survival. It remains unclear whereas these patients should all receive maintenance therapy with it’s toxicity and cost.

Aims: To assess efficacy of maintenance therapy with Bortezomib in patients with MM, who have achieved complete remission after ASCT with MRD positive and negative status.

Methods: From January 2014 to February 2016 52 patients with MM (19 male and 33 female) ages from 24 to 66 years (median 54 years) who have achieved complete remission after ASCT were randomized for a year-long maintenance therapy with Bortezomib. On 100th day after ASCT and after completion of maintenance therapy samples bone marrow from all patients were assessed using 6-color Flow Cytometry to detect MRD. We chose Relapse-Free Survival (RFS) as the indicator of maintenance therapy efficacy. Kaplan-Meier survival curves were compared using log-rank test. Statistical analysis was performed using SAS 9.4.

E1296

LONG-TERM OUTCOME OF MULTIPLE MYELOMA (MM) PATIENTS TREATED UP-FRONT WITH SINGLE OR TANDEM AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) - SINGLE CENTRE EXPERIENCE WITH 334 PATIENTS

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Background: ASCT after induction treatment has been standard of care for MM for almost 30 years. Some centers routinely perform two transplantation up-front (so-called tandem transplants), while others advocate postponing the second transplant until after progression. In recent years novel antimyeloma agents have significantly improved the prognosis of MM patients, thus casting further doubts on the value of the more aggressive tandem ASCT approach.

Aims: To describe long-term outcomes of MM patients treated with ASCT (single and tandem) in a single centre. alled tandem transplants), while others advocate postponing the second transplant until after progression. In recent years novel antimyeloma agents have significantly improved the prognosis of MM patients, thus casting further doubts on the value of the more aggressive tandem ASCT approach.

Methods: This was a retrospective analysis of outcomes of 334 MM patients who underwent 470 ASCT procedures at our center between 1993 and 2014. During that period treatment policies changed from single to tandem to salvage second ASCT, as data from different clinical studies became available.

Results: 296 patients received VAD (vincristine, doxorubicin, dexamethasone) as induction therapy and 38 regimens based on immunomodulatory drugs or proteasome inhibitors. All received high-dose melphalan for pretransplant conditioning, 32 in combination with total body irradiation. Tandem ASCT (defined as second transplantation performed within 6 months after the first) was performed in 136 patients (single ASCT in 168 and salvage second (after relapse/progression) in 30 patients. Transplant related mortality was 1.5%. Median follow up is 70 months (range 4 – 238). Median overall survival (OS) for the entire group is 123 months and median progression free survival (PFS) 40 months. Tandem ASCT in comparison to single and second salvage transplantation resulted in superior OS (203 vs 86 vs 68 months respectively, p<0.0001) and PFS (60 vs 38. vs 25 months respectively, p<0.0001) (figure). Thirteen percent of patients who underwent tandem ASCT are alive and progression-free more than 10 years after the procedure. Fourteen patients developed secondary malignancies.

Figure 1. 2-year Relapse-Free Survival in patients with MRD-negative status after ASCT was higher (p=0.05) than that in MRD-positive patients - 52.9% (95% CI: 35.5-70.5%) vs 37.2% (95% CI: 25.4-49.3%). The MRD-positivity significantly increases the risk of relapse (HR=1.7; 95% CI: 1.2-3.4; p=0.05) Two year cumulative probability of relapse after ASCT in patients with MRD-negative status, who had (n=15) and hadn’t received (n=10) maintenance therapy with Bortezomib was not different (p=0.58). Average time of relapse in MRD-positive patients who received maintenance therapy with Bortezomib was 5 months longer than in the group of patients without maintenance therapy - 17.3 months vs 12.3 months. In the group of MRD-positive patients who did not completed maintenance therapy, relapse was diagnosed in 6 patients. After the end of the treatment 42% of MRD-positive patients achieved MRD-negative status. RFS in this group of patients was significantly higher than in the group of treated MRD-positive patients who retained that status after maintenance therapy (MT) - 100% vs 20% (p=0.02, Fig. 1).

Summary/Conclusions: In cases when MRD-negative status was achieved after ASCT, maintenance therapy does not increase the RFS. In comparison – patients with positive MRD status after ASCT require maintenance therapy to improve their survival rate.
showed an association of EMD with other adverse prognosis factors and unfavorable outcomes. Results: reports evaluating EMD prevalence in pts undergoing autologous hematopoietic stem cell transplantation (aHSCT) are scarce.

Aims: We aimed to evaluate the clinical and laboratory characteristics of pts with EMD as well as its impact in outcomes of MM pts submitted to aHSCT (response to treatment, overall survival [OS] and progression-free survival [PFS]).

Methods: We analysed 155 MM pts submitted to aHSCT in our centre between January/2007 and December/2015, excluding second procedures. The assessment of response to treatment was based in the International Myeloma Working Group consensus criteria (2016).

Results: The median age of the cohort was 58 years (27-69), with 58% of males. The most common subtype of MM was IgG (45%). In our cohort, IgG (29.7%) presented EMD at diagnosis, which was significantly higher compared to reports in the literature (p<0.001; 95% CI 0.22-0.37). The more common involved sites were vertebral column (49%), ribs (13%) and pelvis (13%). EMD occurred more frequently in males (38% vs 18%; p=0.012) and in pts with bone disease (67% vs 50%; p<0.001). Pts with EMD occurred more frequently in males (38% vs 18%; p=0.012) and in pts with bone disease (67% vs 50%; p<0.001). Compared to patients in remission at D100, 82% (p=0.022) and without anaemia at diagnosis (28% vs 11%; p=0.023).

No other significant differences in characteristics at diagnosis were found between pts with and without EMD. Pts with EMD achieved lower complete response/ very good partial response (CR/VGPR) proportions previously to aHSCT (30.4 vs 53.2%; p<0.009), as well as at 100 days after aHSCT (D100) (41.3 vs 59.6%; p=0.037). However, no differences were found concerning refractoriness to first line therapy or proteasome inhibitor (PI) treatment, despite EMD pts received a higher mean number of therapeutic lines previously to aT (1.7 vs 1.6; p=0.1; p=0.023). After a median follow-up of 46.6 months, the median OS was not reached for global cohort and both groups, and there were no differences concerning radiotherapy treatment (72%) or thalidomide maintenance after aHSCT (32%) (p=NS).

Summary/Conclusions: In our cohort, EMD prevalence was significantly higher than usually described in the literature. This observation was probably associated with more carefully surveillance of EMD in aHSCT candidates. EMD was associated with a lower proportion of CR/VGPR previous to aHSCT and at D100 evaluation, even after a higher number of therapeutic lines, although we failed to demonstrate that EMD was an independent prognosis factor for PFS and OS. PI seem also to be the best first-line therapeutic approach for EMD pts. In conclusion, our study suggests that EMD is underdiagnosed in MM pts. It is necessary to achieve a better knowledge of the physiopathology of EMD, in order to define better treatment options that may overcome its negative impact in therapeutic response.

E1298
DIFFERENCES IN PATIENT AND DISEASE CHARACTERISTICS OBSERVED AT INITIATION OF FIRST-LINE AND INITIATION OF SECOND-LINE TREATMENT IN PATIENTS WITH RELAPSED MULTIPLE MYELOMA IN THE CZECH REPUBLIC

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Background: Tools such as the International Staging System (ISS) and the revised ISS (R-ISS) are used to stratify risk of disease in patients with multiple myeloma (MM), enabling assessment of survival expectations. These tools are based on factors measured at diagnosis only; understanding the role of these factors at relapse is less clear. Patient characteristics change with multiple myeloma (MM), enabling assessment of survival expectations. These tools are based on factors measured at diagnosis only; understanding the role of these factors at relapse is less clear. Patient characteristics change with multiple myeloma (MM), enabling assessment of survival expectations.

Methods: We compared the patient- and disease characteristics measured at diagnosis (or available at last follow-up) and at the time of relapse (D100) in 3027 MM patients treated in their first-line (1L) and second-line (2L) treatments. The Registry of Monoclonal Gammopathies (RMG) is a large hematological disease registry, collecting data from patients in the Czech Republic and Slovakia. Data from the RMG can be used to explore real-world characteristics of newly diagnosed MM throughout the disease course.

Aim: To explore how key characteristics of patients with relapsed MM evolve between initiation of 1L treatment and initiation of 2L treatment to better understand drivers of disease progression and death.

Methods: This non-interventional, observational, retrospective study used data collected prospectively from Czech patient charts available in the RMG. Adults (≥18 years old) initiating 1L treatment for MM between May 2007 and April 2016 were included (N=3027); those with smoldering MM were excluded. Patient and disease characteristics were extracted at initiation of 1L and of 2L treatment. Repeated measurements were available only for those who initiated 1L and 2L treatment (1L+2L group; N=1418); patients who did not start 2L treatment may have been in remission, lost to follow-up or had died. Patient and disease characteristics are summarized in the table (all patients starting 1L and those who started 1L+2L). In general, for patients who received 1L+2L treatment, their health status improved between initiation of 1L and of 2L treatment. At 2L, patients tended to have a lower ISS stage (re-measured at 2L) than when they started 1L (stage I at 1L: 26.6%; at 2L: 41.1%). Similarly, the proportion of patients with R-ISS stage III disease was lower at start of 2L (24.6%) than at start of 1L (31.1%); Eastern Cooperative Oncology Group performance status scores were also better for patients when they started 2L than when they started 1L (stage 3-4 at 1L: 14.7%; at 2L: 5.5%). Laboratory measurements indicated that patients were in better health at the start of 2L treatment than at initiation of 1L treatment: median M protein levels decreased from 31.2 g/L at 1L to 17.7 g/L at 2L, and elevated calcium and creatinine levels were less common at 2L than at 1L. Median lactate dehydrogenase levels were slightly elevated at start of 2L vs start of 1L treatment (184.4 U/L vs 206.6 U/L).

Summary/Conclusions: Patient health was better at initiation of 2L treatment than at initiation of 1L treatment. At relapse, patients are likely to be closely monitored and are able to initiate the next treatment line while in relatively good health; at initiation of 1L, patients may have experienced deterioration in health which could have triggered their diagnosis. These findings illustrate how patient characteristics change at relapse and that the underlying disease survival may evolve; therefore, restaging patients at relapse may be beneficial and could contribute to improved predictive tools that can better define survival estimations at first relapse by considering patients’ experiences at 1L.

E1299
AN EARLY GOOD RESPONSE AFTER BORTEZOMIB-BASED INDUCTION REGIMENS REPRESENTS A SIGNIFICANT PREDICTOR FOR IMPROVED PFS IN NDMM PATIENTS

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Background: Introduction of triplets-based induction regimens containing proteasome inhibitors (PIs) in clinical practice have led to higher response rates and prolonged life expectancy in newly diagnosed multiple myeloma (NDMM)
patients. Different studies have linked complete response (CR) with better PFS (progression free survival), but not always with prolonged overall survival (OS), most likely due to the impact of novel agents in the management of relapsed-refractory patients. Overall, these observations suggest PFS as a more reliable predictor of clinical outcome. Also, the biological aggressiveness is emerging as a pivotal disease characteristic which affects clinical behavior and response to therapy. In this context, little is known about the association of response kinetic with survival outcomes.

**Aims:** In order to evaluate whether early achievement of a good quality response impacts on outcome, we retrospectively analyzed 87 NDMM patients treated at our institution with bortezomib containing regimens (BRs).

**Methods:** From 2004 to 2016, 87 patients with NDMM and measurable disease (serum and/or urine M protein) were treated with BRs. Both patients eligible and non-eligible to ASCT were included in the study; patients undergoing ASCT were censored at the time of transplant. Median age was 66 (range 32-87); males were 51 (59%); 72 (83%) patients were in Ill stage; median follow up was 30.7 months; median number of administered courses was 5 (range 2-9).

PFS was defined according to IMWG criteria. Cytogenetic risk evaluation performed by a standardized FISH panel, including del17p, del13q, t(11;14), t(4;14), was available in 37 patients (42.5%). Among these high risk abnormalities were identified in 20 patients. Early good response (EGR) defined an M protein reduction ≥75% after 2 courses of therapy. Survival curves were calculated for PFS and OS by Kaplan Meyer method, using log-rank test.

**Results:** PFS and OS were both assessed in patients who achieved EGR as well as in patients who did not. No significant differences were observed in terms of OS between the two groups, whilst PFS was significantly longer in patients achieving EGR (p = 0.036, median PFS 44.7 vs 29 months, respectively). Next, we analyzed patients with high risk cytogenetic. Among these, EGR vs non-EGR patients reached a PFS of 43.7 and 18.7 months, respectively. Remarkable PFS differences between these two groups were not significant (p value=0.11).

**Summary/Conclusions:** Overall, our data demonstrate a significant impact of EGR on PFS in NDMM patients after BRs, irrespective of median age at diagnosis. In presence of high cytogenetic risk EGR is associated with prolonged PFS, although not significantly. Ongoing analysis on larger cohort of high risk patients will confirm the impact of EGR on PFS also in this group of patients. Based on our data kinetic of response, deriving from EGR assessment, may provide information on both disease aggressiveness as well as clinical outcome, thus representing a novel, surrogate marker for an early survival analysis, with favorable cost-effectiveness characteristics. In summary biological and clinical information deriving from EGR analysis combined with cytogenetic risk evaluation and patient-related (age, comorbidities) characteristics, may represent a useful tool to make clinical decisions. Further prospective evaluations are needed to include this marker in clinical practice.

**E1301**

**RELATIVE PROGRESSION-FREE SURVIVAL OVER TIME OF NOVEL TRIPLET REGIMENS FOR THE TREATMENT OF RELAPSED/REFRACTORY MULTIPLE MYELOMA**

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**Background:** In combination with lenalidomide (REV/LIMID®) and dexamethasone (d), elotuzumab (EMPLICITI™, E), carfilzomib (KYPROLIS®, K), and ixazomib (NINLARO®, N) were recently approved for the treatment of relapsed/refractory multiple myeloma (RRMM). In randomized controlled trials, all three drugs showed a significant relative reduction in the risk of disease progression or death as compared to patients who received Rd. To date, there have been no head-to-head trials comparing ERd, KRd, and/or NRd.

**Aims:** To describe the time-specific progression-free survival (PFS) based on published Kaplan-Meier PFS curves for ERd, KRd, and NRd relative to Rd.

**Methods:** Individual patient-level data (IPD) were reconstructed from the published Kaplan-Meier PFS curves from the ELOQUENT-2 (ERd), ASPIRE (KRd), and TOURMALINE-MM1(NRd) randomized, controlled, Phase III trials using digitization software and the methods described by Guyot, et. al. Using the reconstructed IPD, Kaplan-Meier survival curves were estimated for each arm within each trial. PFS curves were digitized by two independent researchers and the reconstructed curves were overlaid with the published data to validate the IPD. In each trial, the relative PFS benefit over time was calculated as the difference in the Kaplan-Meier PFS estimate of each triplet regimen and the Kaplan-Meier PFS estimate of Rd divided by the Kaplan-Meier PFS estimate of Rd: rPFS(t)=(S_{ERd}(t) - S_{Rd}(t)) / S_{Rd}(t). Where S(t) denotes the Kaplan-Meier PFS estimate at time t, and X denotes E, K, or N, respectively.

**Results:** IPD from the three randomized controlled trials was successfully reconstructed and validated. Numerically, ERd had the highest relative PFS over the initial 10 months of treatment and showed sustained benefit from month 24 onwards (Figure 1). At 12 months, the relative PFS benefit was 17.9% for ERd, 21.7% for KRd, and 9.7% for NRd. At 24 months, the relative PFS benefit was 45.1% for ERd, 34.3% for KRd and 24.1% for NRd. At 36 months, the relative PFS benefit was 39.9% for ERd and 19.1% for KRd. ERd had a higher relative PFS than NRd for almost the entirety of RRMM treatment. At the end of data availability, NRd and KRd showed no additional PFS benefit relative to Rd, while ERd showed a sustained benefit through 40 months. Data will be updated for the conference, where available.

**Figure 1.**

**Summary/Conclusions:** For the treatment for RRMM, ERd showed an early and sustained benefit in relative PFS which was maintained through 40 months. KRd and NRd showed initial benefits which faded by the end of data availability.
Results: A total of 82 patients (median age 68 years [range: 43-88]) were included in this analysis. CRAB criteria included anemia in 23 patients (28%), renal insufficiency in 8 (9.8%), hypercalcemia in 13 (16%) and bone lesions in 54 (66%). Median time from diagnosis to start pomalidomide was 5.75 years [range: 0.8-18.4], median number of treatment cycles was 3 [range: 1-17]. At time of analysis 59 patients had stopped pomalidomide treatment: 24 patients had progressive disease, 10 had unacceptable toxicity, 6 patients were refractory, 4 patients died during treatment and 15 patients stopped due to various other reasons. Grade ≥3 hematological adverse events occurred in 11% of patients, 4% had neutropenic fever. Grade ≥3 non-hematological toxicities occurred in 57% of patients, including infection in 22%, gastrointestinal disorders in 5% and renal disorders in 5%. Of 69 patients evaluable for response ORR was 41%, with a partial response (PR) rate and a very good partial response (VGPR) rate of 29% and 3%, respectively. Response based on age was not significantly different (p=0.426). Median PFS for all patients was 3.8 months (95% confidence interval [CI] 2.3-6.6), Patients >65 years had a longer median PFS of 5.7 months (95% CI 2.8-8.0) versus 2.8 months (95% CI 1.9-6.6) in patients ≤65, however, this was not statistically significant (p=0.426) (figure 1). For patients achieving ≥PR, median PFS was 9.6 months (95% CI 5.7-not reached [NR]), as compared to 2.2 months (95% CI 1.9-6.6) among patients treated within 5 years after diagnosis (p=0.05). Data about previous treatment, ISS stage, cytogenetics at diagnosis and an update of OS will be presented at EHA.

Methods: We prospectively followed 44 MM transplanted patients: 19 with IgG-kappa isotype, 11 with IgG lambda, 9 with IgA-kappa and 5 with IgA-lambda. They were followed for 29.0±3.8 months (mean±standard error [SE]). Serial serum samples from each MM patients were collected periodically after APBSCT. Relapse or progression was defined according IWG criteria. To identify factors that predict disease progression in MM transplanted patients, we studied hematologic/light chains (HLC) pair quantification, sFLC and total immunoglobulins levels in serial serum samples collected during the follow-up. Involved/uninvolved index (I/Ui) was calculated using the monoclonal chain (Involved) as numerator and the polyclonal chain of the same class (Uninvolved) as denominator. The HLC ratio (HLC) was calculated as IgGk/IgGλ or IgAk/IgAλ with normal reference ranges established in 1.3-3.7 for IgG and 0.7-2.2 for IgA.

Results: In IgG MM patients, values of I/Ui were significantly increased in pre-relapse compared to basal samples (8.49±4.01 vs 2.23±0.67 p=0.012). By contrast, this index remained stable along follow-up in patients in complete remission (CR) or with a partial response (PR). However, the later showed higher values of I/Ui ratio, suggesting that the presence of an M-component induces immunosuppression of the uninvolved chain in patients with monoclonal protein (MC) or free light chains (sFLC). The identification of new biomarkers to early predict P/R might be clinically useful for an anticipated treatment.

Summary/Conclusions: Our results show that HLC-pair measurement could detect progression or relapse and the increase of MC in transplanted MM patients earlier than other methods. Future studies will need to demonstrate the real value of the I/Ui index as a biomarker to anticipate progression in MM patients subjected to APBSCT.

E1303 MULTIPLE MYELOMA IMMUNOPHENOTYPIC REMISSION IS A SIGNIFICANT PREDICTOR OF PROGRESSION FREE SURVIVAL AFTER FIRST AUTOLOGOUS STEM CELL TRANSPLANTATION - PILOT STUDY

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Background: Minimal residual disease in multiple myeloma assessed by multiparameter flow cytometry has become an increasingly important predictor of progression-free survival (PFS).

Aims: Our primary endpoint was to evaluate PFS in myeloma patients after stem cell transplantation who reached immunophenotypic CR (iCR) versus those who have not.

Methods: We prospectively evaluated prognostic importance of minimal residual disease detection by multiparameter flow cytometry (MFC) in multiple
myeloma patients who underwent autologous stem cell transplantation from January 2014 until December 2016. All patients were uniformly treated with bortezomib based induction therapy followed by high dose chemotherapy (Melphalan 200mg/m²) and autologous stem cell transplantation. Minimal residual disease (MRD) status was determined by β-colour MFC 1 month after autologous transplantation from bone marrow aspirate in all patients who achieved at least conventional VGPR or CR.

Results: We identified 56 patients who fulfilled the above mentioned criteria. 30 were males and 26 females, median age was 61. 62.5% of patients (35/56 patients) achieved CR, 37.5% of patients (21/56) did not. Median follow up of the cohort was 19 months (6-59), 32.1% of patients (18/56) relapsed during the follow-up period. 16.1% of patients (9/56) died, 22.9% (13/56 patients) in CR and 47.6% (10/21 patients) not in CR relapsed during the follow up. Patients in iCR showed significantly longer PFS with median 42 months than those in less than iCR with PFS median 29 months (p=0.0196, log-rank test).

This was associated with a hazard ratio of relapse (0.3565) for iCR group.

Summary/Conclusions: Achieving immunophenotypic CR is clearly associated with longer progression free survival compared to conventional CR. Reaching iCR should be a goal of myeloma treatment.

E1304

REGULATION OF NORMAL AND MONOCLONAL IMMUNOGLOBULIN SECRETION BY CYTOKINES (S- SYNDECAN-1, BLYS & TGF-BETA-1) IN PATIENTS WITH IG-SECRETING B-CELL DISORDERS AT PRESENTATION.

PROGNOSTIC IMPLICATIONS

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Background: The most common neoplastic lymphoproliferative diseases that secrete paraprotein are multiple myeloma (MM), Waldenstrom’s Macroglobulinemia (WM) and chronic lymphocytic leukemia (CLL). The two first entities secrete paraprotein by definition, while serum free light chains (sFLC) are increased in 50% of CLL cases. Microenvironmental factors, such as soluble Syndecan-1 (ssynd-1) and BlyS normally promote lymphoplasmacytic differentiation as well as their secretory activity, whereas others, like TGFbeta1, inhibit polyclonal Igs, both being reflected by the corresponding ratios (HLCR).

Methods: We studied 269 patients: 105 with MM (79 IgG and 26 IgA, of whom 33%, 31%, and 36% were stacked ISS 1, 2 and 3 respectively), 64 suffering from WM (44%, 28%, 28% staged WM-IPSS 1, 2, 3 respectively), and 100 with CLL (67%, 23%, 10% staged Binet 1, 2, 3 respectively). Patients were regularly followed up since last visit or since diagnosis (median follow up 6 months). sFLC/sFLCR and HLC/HLCR were determined by nephelometry (Freelite™ and Heavyvite™, the Binding Site Birmingham, UK) while ssynd1, BLYS and TGFbeta1 by ELISA, either in fresh or in frozen sera sample drawn at the time of diagnosis. Statistical analysis was performed by standard methods using the SPSS v22.0, software.

Results: The main correlations observed between the Ig levels secreted in the 3 diseases and cytokines studied, as well as their impact with regard to patients’ outcome, are shown in table.

Summary/Conclusions: ssynd1 in MM and BLYS in WM and CLL correlated with progressive disease. By inhibiting both monoclonal and polyclonal Ig, TGFbeta1 correlated with MM in both HLC and FLC ratios and differences. In addition, the aforementioned variables are prognostic with regard to patients’ outcome.

E1305

PATIENTS WITH MULTIPLE MYELOMA (MM) IN LONG TERM COMPLETE REMISSION (LTRC) AFTER AUTOLOGOUS TRANSPLANTATION (APBSCT) EXPRESS A DISTINCTIVE INMUNE PROFILE WITH POTENTIAL PROGNOSIS VALUE

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Background: A small fraction of patients with MM could be considered potentially cured as long as they remain for more than six years in long term complete remission (MM-LTRC) after autologous transplantation (APBSCT). The exhaustive study of the immune status of these patients could highlight interesting information.

Aims: Here we present an observational study that evaluates the numbers and phenotype of T- and B-cells subsets in the peripheral blood (PB) of MM-LTRC patients. This study could determine if differences in the immune status between healthy adults and MM-LTRC patients exist, and if so, what are the specific alterations.

Methods: After approval by the ethics committee, we selected 13 patients diagnosed with MM, in sCR according to IMWG criteria for at least six years after APBSCT, and 15 healthy adults (HA) of similar ages as a comparative group. Group MM: 7 males and 6 females; median age: 61. Median follow up (sCR) was 8 years (range 6-19). Group HA: 5 females and 10 males, median age 60 (36-78). Immunephenotype characterization was done using a comprehensive 8-color flow cytometry panel. Subpopulations of CD4+ and CD8+ T-cells from PB were quantified, including naïve, central and effecter memory, regulatory T-cells, as well as subpopulations of B-cells: naïve, transitional, marginal zone-like, class-switched memory and plasmablasts.

In order to confirm their specific immune signature, the analysis was repeated in the same LTRC-MM patients one year after the first analysis was done. A Kruskal-Wallis test was used to evaluate differences among the studied groups. A posteriori test was done to compare the control group with the two patient’s group (patients and patients +1 year), independently of each other. A Wilcoxon matched test was used to compare a patient under group “patients” with the status of the same patient in the second group “patients +1 year”. Statistical analysis was done using GraphPad Prism software.

Background: Patients have a lower percentage of total CD4+ T-cells (p=0.0004) together with a decrease in the naïve CD4+ T-cells (CD27-CCR7+CD45RA+; p=0.0004) and an increment of the effector memory CD4+ T-cells (CD27-CCR7+; p=0.0028), both CD27-CCR7+CD45RA+ and CD27-CCR7+CD45RA+ similar results were found within the CD8+ T-cells. No differences were observed in the Th1 defining CD4+CD25+IFN-CD127. The mean percentage of total B-cells in the patients was within the normal range and no significant differences were found when compared to HA. However, naïve B-cells (CD27-IgD-IgM+) proportion was higher in patients and a corresponding reduction of marginal zone-like B-cells (CD27-IgD+IgM+; p=0.0047) and class-switched memory B-cells (CD27-IgD+IgM+; p=0.0043) was observed. No differences were observed in the percentage of transitional B-cells (CD27-CD10+CD38+) or plasmablasts (CD27++ CD38++) in the PB of the two groups. When the analysis was repeated in the same LTRC-MM patients one year after the first analysis, no changes were detected neither when analysed as a group nor when analysed individually.

Summary/Conclusions: The MM-LTRC patients seem to express a distinctive immune “footprint” characterized by a decreased proportion of naïve T-cells and an increased percentage of effector T-cells, which probably exert a competent immune surveillance. Conversely, the increase in naïve B cells may guarantee the humoral response host immunity, inhibiting the most harmful tumor plasma cells that might compete with myelomatous cells for normal bone marrow niches. The precise role of these refined immunological studies in the monitoring and therapeutic decisions in MM patients, and also in the duration of sCR, should be defined in the future.

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E1306

IMPACT OF THE AFFORDABILITY OF NOVEL AGENTS IN PATIENTS WITH MULTIPLE MYELOMA: REAL WORLD DATA ON CURRENT CLINICAL PRACTICE IN MEXICO

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E1307
BASAL CALCIUM, AN IMPORTANT ELEMENT IN THE DEVELOPMENT OF CALR MUTANT MPNS
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Background: Calreticulin (CALR) is a calcium (Ca2+) buffering chaperone mutation of which has recently been associated with essential thrombocythemia and primary myelofibrosis without JAK2 mutations. These mutations have been suggested to impair the Ca2+ buffering activity of Calreticulin due to a change of the negative charge in its C-terminal domain. Ca2+ is known to be important during megakaryocyte activity; however its role during megakaryopoiesis and the possible link of CALR mutations and abnormal megakaryocyte production due to impaired Ca2+ buffering activity in myeloproliferative neoplasms (MPNs) remains unclear.

Aims: Here we aim to understand how basal Ca2+ fluctuations during normal megakaryopoiesis and how CALR mutations could affect the basal Ca2+ levels in megakaryocytes in MPNs.

Methods: Ca2+ staining was performed using Fluor-8 dye and Ca2+ basal levels were measured by flow cytometry. Changes in basal Ca2+ during megakaryopoiesis using two cellular systems, K-562 cells and mouse bone marrow cells, were measured each 24 hours. Further studies using CALR mutant cellular models were performed using the same methodology.

Results: Our results showed a characteristic behaviour of fluctuations of basal Ca2+ during this megakaryopoiesis, where Ca2+ levels decrease in the last stage of megakaryocyte formation. These results suggest that Ca2+ reduction could be essential for megakaryopoiesis. In order to understand how CALR mutations affect basal Ca2+, Marimo cells and Dami cells expressing CALR mutations were analysed. Here we show a decrease in basal Ca2+ in Marimo cells and Dami-CALR type2 mutation compared to the controls. Moreover, Dami-CALR type1 did not show any significant reduction, suggesting possible differences in Ca2+ behaviour depending in CALR type mutation. We are currently working in the analysis of basal Ca2+ fluctuations during megakaryopoiesis in the presence of CALR mutations and preliminary results show abnormal basal Ca2+ levels throughout all the process of megakaryocyte differentiation.

Summary/Conclusions: Altogether, our findings indicate that basal Ca2+ could be an important element during megakaryopoiesis and CALR mutations found in MPN could impair the normal production of megakaryocytes due to changes in cellular Ca2+. However, further analysis need to be done in order to understand the role CALR mutations and their effect in the Ca2+ buffering activity of CALR in MPNs.

E1308
THE INHIBITION OF JAK/STAT SIGNALING IS COMPENSATED BY ACTIVATION OF MAPK PATHWAY IN MYELOPROLIFERATIVE NEOPLASMS
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Background: Myeloproliferative neoplasms (MPN) remain incurable regardless of advancement in the use of JAK1/2 inhibitor Ruxolitinib, which competence is unrelated to the JAK2V617F mutation.

Aims: We want to explore JAK1/2 inhibition dependency in correlation with activated JAK/STAT3 signaling and cell cycle in MPNs.

Methods: The immunoblotting has been used to analyze activation of JAK/STAT3, PI3K/AKT and MAPK signaling in JAK2V617F mutated HEL cells and granulocytes of MPN. The cell cycle and apoptosis of granulocytes are studied by flow cytometry.

Results: Concerning myeloproliferation, JAK1/2 inhibitors reduced the percentage of cells in G2M phase and increased apoptosis in JAK2V617F mutated HEL cells. Comparing to polycythemia vera (PV), the percentage of granulocytes is decreased in S and G2M phases of essential thrombocythemia (ET) and primary myelofibrosis (PMF) that demonstrated increased apoptosis.

Hyperbromocyclhexane increased percentage of granulocytes in GoG1 phase of JAK2V617F positive, but reduced in JAK2V617F negative PMF, the later one similar to Ruxolitinib. JAK1/2 inhibitors reduced percentage of apoptotic granulocytes in JAK2V617F positive, but increased in JAK2V617F negative PMF. JAK1/2 inhibitors could not impair constitutive activation of JAK/STAT3 signal in HEL cells as well as in granulocytes of JAK2V617F positive ET and PMF. Absence of JAK2V617F mutation supported dephosphorylation of JAK/STAT3 pathway by JAK1/2 inhibitors in ET, but not in PMF. JAK1/2 inhibitor
Ruxolitinib largely activates MAPK signaling in MPN, while slightly PI3K/AKT signaling in PV and JAK2V617F negative PMF. Specific JAK2 inhibitor Hexabromocyclodexane activates PI3K/AKT signaling in JAK2V617F positive ET, but reduced in JAK2V617F negative ET and PMF.

**Summary/Conclusions:** This observation support cross-talk between examined pathways, where inhibition of JAK/STAT3 signaling is compensated by activation of MAPK pathway irrespective of JAK2V617F mutation, while PI3K/AKT signaling demonstrates JAK2V617F dependence in MPN.

**E1309**

**CIRCULATING PLATELET AND MEGAKARYOCYTE-DERIVED MICROPARTICLES OF JAK2V617F MUTATED PATIENTS WITH MYELOFIBROSIS ARE DISTINCTLY MODIFIED: A NOVEL LIQUID BIOPSY TOOL OF RESPONSE TO RUXOLITINIB?

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**Summary/Conclusions:** Essential thrombocythemia (ET) is a myeloproliferative neoplasm (MPN) characterized by a sustained elevation of platelet counts. Patients are at risk of hemorrhagic or thrombotic complications, and, eventually, of progression to acute myelogenous leukemia or marrow fibrosis. ET patients are classified on genetic subgroups based on known driver mutations (i.e. JAK2V617F, MPL W515K/L, CALR Type III). So-called triple-negative patients (TN) do not bear any of the aforementioned mutations but may show hitherto unknown mutations that have not been hitherto shown to be causative of the disease. Cytoreductive or anti-platelet treatment is currently being used in the clinic. However, a comprehensive study of platelet properties is lacking in these patients, with updated genetic classification.

Aims: to characterize platelet of ET genetic groups and establish a phenotypic and functional profile which will help us to understand the pathophysiology of this disease and potentially add a better patient diagnosis/prognosis.

**Methods:** More than 40 ET patients (from Belgian and Spanish cohorts) and healthy donors (HD) were recruited. Since treatment with acetylsalicylic acid is common, a number of HD that had taken the drug 3 consecutive days prior blood sampling were recruited. Platelets were subjected to a functional assay (Platelet aggregation) and flow cytometry analysis of surface marker expression. A novel flow cytometry based platelet aggregation assay (de Cuypet et al, Blood 2013) has been used to measure kinetics and quantitate the responses to different platelet receptors (CLEC2, GPIIIb/IIA, WRF R, and collagen receptors GPVI and GPIIb/IIIa) upon specific agonist stimulation.

**Results:** Among the TN cases we identified four MPL S204F/P cases that were analyzed separately given that part of their hematological parameters (MPV, RBC counts) were not similar to the rest of the ET cases. Additionally, flow cytometry analysis also showed that MPL S204F/P platelets are larger and have altered expression of surface markers (CD62P, CD41a in TN compared to the other ET groups and HD. On the other hand, JAK2 V617F and CALR type I ET platelets exhibited normal to increased expression density of these receptors as compared to HD. Variable patterns were observed amongst the ET genetic subgroups, with reduced responses especially upon challenge with Aggrelin A or collagen, while platelets from the JAK2 ET subgroup displayed a high reactivity to collagen. JAK2 V617F and Ruxolitinib® changed the activation pattern of these patients. When specific functional and phenotypic platelet pattern established they could contribute significantly to a better diagnosis/prognosis of the disease.

**E1311**

**ASSOCIATION ANALYSIS OF CYTOGENETIC AND GENETIC ALTERATIONS IN PRIMARY MYELOFIBROSIS**

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**Background:** A number of genomic abnormalities have been associated with primary myelofibrosis (PMF). Next generation sequencing (NGS) and single-nucleotide polymorphism array (SNP-A) methods are used for PMF genomic studies and certain cytogenetic and genomic associations have been determined. To better characterise the genomic landscape of PMF we performed comprehensive analysis of gene mutations and chromosomal aberrations in a population-based cohort of PMF patients.

**Aims:** Characterize genomic aberrations in MPN using SNP-A and NGS methods.

**Methods:** PMF peripheral blood samples were screened by Infinum HD whole-genome genotyping assay with the HumanCytosNXP-12 BeadChips (Illumina Inc., CA). NGS analysis was performed using TruSight Myeloid 54 gene target panel (Illumina). SNP-A and NGS data analyses were performed using Illumina BaseSpace Informatics suite (Illumina). JAK2, CALR, MPL mutations were additionally confirmed with Sanger sequencing while small indels – with DNA fragment analysis.
Results: 110 patients diagnosed with PMF according to WHO criteria between years 2013 and 2014 were included into the study. SNA analysis identified 77 chromosomal abnormalities in 61 patients (55.4%). These comprised the loss of heterozygosity (LOH) (59.7%), hemizygous deletions (23.4%) and copy number gains (16.9%). The most common aberrations in affected patients were: 4q loss (55.7%), 20q deletion (11.5%), 1q duplication (4.9%), 19p deletion (4.9%), 1p loss (3.2%) and 6q loss (3.2%). NGS analysis detected 219 gene mutations (in a total of 27 gen) in 108 patients (98%). The most frequently mutated genes were: JAK2 (62.9%), CALR (27.8%), ASXL1 (20.3%), JAK2 exon 12 mutation (16.6%), MPL (7.4%), <5% ZRSR2, EZH2, DNMT3A, U2AF1, ET6V, SF3B1, IDH1, IDH2. Recurrent specific mutations were detected in 10 gen. Sixty-two patients (57.4%) had more than one somatic mutation. Six patients (5.5%) had no JAK2, CALR or MPL mutations and were defined as “triple-negative”. Previously not described SRSF2 gene 12 bp insertion was indentified in four patients (3.7%). The correlation analysis showed significant associations between 9p LOH and JAK2(V617F)mutation (p=0.011), 19p deletion and CALR mutations (p=0.004). Notably, the affected genes are located in core-sponding affected chromosome regions, indicating disruption of both alleles by different biological mechanisms. KRAS and ET6V mutations were statistically associated with ASXL1 mutations (p<0.001 and p=0.005, respectively) while JAK2 and CALR mutations were mutually exclusive in all cases (p<0.001).

Summary/Conclusions: A number of associations between gene mutations and chromosomal aberrations was revealed in PMF. Co-presence of 9p LOH with JAK2(V617F) and CALR mutations with 19p deletion indicate that further deregulation of these key signaling pathways may take place disrupting the second allele of the affected genes by different biological mechanism – LOH or deletion.

E1312

FREQUENCY OF CONCURRENT BCR-ABL1, JAK2, CALR AND MPL MUTATIONS IN A COHORT OF 5,545 CASES WITH SUSPECTED MPN BY A DEEP SEQUENCING APPROACH

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Background: Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm characterized by BCR-ABL1, whereas in about 90% of BCR-ABL1-negative MPN a mutation in CALR, JAK2 or MPL can be detected. These genetic alterations are thought to be nearly mutually exclusive, however, an accurate frequency is still missing.

Aims: To determine the incidence of genetic markers occurring in parallel in a large cohort of patients with suspected MPN and characterize double mutated cases.

Methods: From July 2016 till January 2017 3545 samples were sent to our laboratory with suspected MPN. The male:female ratio was 1:1, and the median age was 60 years (range: 18-98 years). Median white blood cell count was 9x109/L, hemoglobin level (Hb) was 15g/dl, and platelet count was 328x109/L. All of these cases were analyzed by an amplicon deep sequencing approach for mutations in JAK2 (exon12, exon14), CALR (exon9) and MPL (exon10) with a sensitivity of 1%. 3070 patients were additionally screened for BCR-ABL1 fusion by a multiplex PCR approach. Samples that were double mutated for JAK2, CALR and MPL were analyzed by amplicon deep sequencing for additional mutations in 13 myeloid genes.

Results: In total 1775/5545 (32%) of suspected MPN patients showed JAK2, CALR and or MPL mutations. 1438 (26%) were JAK2, 267 (5%) CALR, and 89 (1%) MPL mutated. Of note, the analysis of a subgroup (n=3070) for BCR-ABL1 fusion identified 123 (4%) as CML cases. The JAK2 mutated cases presented mainly with Va181T7Phe (99%) and rarely with JAK2 exon12 mutations (1%). CALR mutations were primarily type 1 (54%) and type 2 (30%). MPL mutations were located at amino acid Trp515 in 96% of cases. Double mutated cases were present in 19/1775 (1%) cases: JAK2/MPL (63%), JAK2/CALR (32%) and CALR/MPL (6%). In nearly all CALR mutated cases (67%) the CALR mutation was detected with the higher load, whereas in JAK2/MPL double mutated cases the ratio was equal. Most of the patients (18/19) had one mutation with a load below 10% and could have been missed by other approaches. BCR-ABL1 together with JAK2 or CALR mutation was found in one patient, each. The remaining 324 patients out of the 2671, overall being prognostically detrimental for survival. CALR mutations had a favorable impact on survival with borderline significance (HR 0.3, p=0.052).

F1313

A COMPREHENSIVE ASSESSMENT OF MOLECULAR AND CYTOGENETIC MARKERS OF PROGNOSIS IN PATIENTS WITH PRIMARY MYELOFIBROSIS

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Background: According to recent reports the data of molecular and cytogenetic analysis (type or absence of driver mutation (DM), mutations in ASX1L, EZH2, IDH/12 genes, karyotype) is a promising tool for prediction of survival in primary myelofibrosis (PMF). Multiple combinations of genomic aberrations lead to clinical course and survival heterogeneity. The aforementioned factors need to be considered together to evaluate their mutual influence.

Aims: The aim of the study was to evaluate a prognostic impact of DM, mutational status of epigenetic regulator (ER) genes, karyotype and their combinations for overall survival (OS) in PMF patients.

Methods: We have examined 110 patients (pts) with PMF (34.5% males). Median (Me) age was 59 years (16-82). For all pts the detection of JAK2V617F was done. JAK2(-) samples were tested for MPL 515 codon mutations and exon 9 mutations of CALR (direct sequencing). All pts except 4 underwent the analysis of mutations in ASX1L, EZH2, IDH1/2 genes with high resolution meltig method followed by sequencing of probably mutated samples. Karytype research was done for 46 (43.6%) pts.

Figure 1.

Results: DM were detected in 81.8% pts: JAK2(+)- 50%, CALR(-)+ 25.5%, MPL(+)- 6.4% cases. No DM were found in 18.2% pts considered triple-negative (TN). Mutations in ER genes were detected in 20.8% pts. High risk (HR) chromosomal aberrations (Chy) unfavorable CA-MKL, tang DIPSS alpha muta-

den:6(q)15), add(6)(p25), del(10q)(p22), t(7;12)(p11;q11) were found in 27.1% pts. Univariate analysis identified HR karyotype (hazard ratio (HR) 8.2, p<0.001), the absence of DM (HR 8.1, p<0.001) and nonsense and frameshift (hereinafter mut) (HR 2.9, p=0.018) but not missense mutations of ASX1L (p=0.378) as being prognostically detrimental for survival. CALR mutations had a favorable impact on survival with borderline significance (HR 0.3, p=0.052). A multivariate analysis included TN, CALR, ASXL1 status and karyotype as covariates revealed an inter-independent prognostic value of HR karyotype (HR=7.4, p<0.001) and ASXL1 mut (HR=2.8, p=0.023). In Cox regression model considering of same covariates except karyotype TN status (HR=2.4, p=0.050) and ASXL1 mut (HR=3.3, p=0.012) but not CALR mutations (HR=0.3, p=0.075) were significant for OS. CALR mutations became significant (HR=0.3, p=0.075) when only ASXL1 mut were included as covariate (HR=3.9, p=0.004). When comparing groups divided by CALR status the shortest OS was noted in CALR(-)/ASXL1+ mut (HR=0.075, p=0.007) in 5 years, p=0.022 CALR(-)/ASXL1+ wider type (wt) pts seem to have better OS than CALR(-)/ASXL1wt (median not reached (with follow up period of 10.1 years) and 13.5 years, respectively, p=0.124). Median OS estimated in pts due to presence/absence of DM and ASXL1 status
was 0.9 years in TNASXL1mut. 3.6 years in TNASXL1wt. 13.8 years in DM(+)+ASXL1twt and was not reached in DM(+)+ASXL1tmut (with follow up period of 10.3 years) group (p<0.0001). Differences in OS depending on the ASXL1 status were statistically significant in the TN group (p=0.007) but not for DM(+) group (p=0.786). The better OS was observed in ASXL1wt pts with low risk (LR) karyotype (Me 6.4 years, p=0.0005). There were no differences in OS of ASXL1wt- HR, ASXL1tmut-LR and ASXL1tmut-HR pts (1.4 vs 1.6 vs 1.2 years, p=0.493).

Summary/Conclusions: The better OS was observed in HR, status were statistically significant in the 10.3 years) group (р<0,0001). Differences in OS depending on the DM(+)ASXL1wt

Results: The JAK2 46/1 haplotype (GG and CG) was present in 170 patients (80.6%) with MPN, in 25 (52%) patients with suspected MPN, in 23 (49%) asymptomatic JAK2 V617F+ patients and in 42 (42%) cases of control group. G variant of rs10974944 was more frequent in all JAK2 V617F+ positive MPN, than in the control population (χ²=24.6, p=0.0001). These results were similar to findings of previous studies, which have shown that the 46/1 haplotype pre-disposes to the acquisition of JAK2 V617F mutation. JAK2V617F allele burden was significantly higher in patients with PV than in patients with ET (p=0,001), but no differences were observed with from patients with the PMF. 46/1 haplotype was closely associated with MPN patients if the allele burden exceeds 5% (Fig. 1) regardless of the phenotype or the treatment. In this case with an increase in JAK2V617F allele burden the JAK2 46/1 haplotype frequency significantly increased. However, there was no significant difference in the JAK2 46/1 haplotype frequencies between patients with allele burden less than 5% and the control group.

Summary/Conclusions: No significant differences of the carrier haplotype frequencies between control group and patients with minimal allele burden (less than 5%) JAK2 2617F have been observed. This is evidence against primary “hypermutability” hypothesis. A further increase in allele load is more pronounced in carriers of haplotype 46/1 that supports the “fertile ground” hypothesis. We hypothesis that DNA mutation JAK2V617F repair is down-regured in 46/1 haplotype carriers.

Background: Several research groups have determined that the JAK2 46/1 (GGCC) haplotype in multiple ethnic groups is strongly associated with a pre-disposition to acquiring JAK2 V617F-positive MPNs. The role of the JAK2 46/1 haplotype in the natural evolution of the mutant JAK2V617F allele burden in PV but not ET or PMF has been shown [Alvarez-Larrán A e.a. Leukemia 2012; 36(3):324-326]. However, the data on the impact of the haplotype on the JAK2 V617F allele burden do not always agree. Using a highly sensitive test allowed to reveal a high prevalence JAK2 V617F among persons without symptoms of hematological disorders [Krichevsky S e.a. Blood Cells, Molecules and Diseases, doi: 10.1016/j.bcmd.2017.01.001]. Influence of haplotype 46/1 for such cases is not known. There are two competing hypotheses of “hypermutability” and “fertile ground” explaining the causes of the higher frequency of mutations of JAK2V617F in haplotype 46/1 carriers. The “hypermutability” hypothesis refers to an increased risk of a primary mutation in carriers of haplotype 46/1. In this case, the increasing frequency of the haplotype in patients with low allele burden (<5%) must also be observed, including those individuals without evidence of hematological disorders.

Aims: Studying the relations of haplotype 46/1 and JAK2 V617F allele burden

Methods: The diagnosis of chronic myeloproliferative neoplasms was based on the WHO (2008) criteria. The cohort included patients with JAK2 V617F mutation: 100 patients with PV, 51 with ET, 14 with MF, 41 patients with unclassified AML). The control group included 100 healthy donors without JAK2 V617F mutation. SNP genotyping of two 46/1 tag SNPs, rs12340895 and rs10974944 (Promega, USA) and were also analyzed for JAK2V617F mutation by PowerPlex System samples were investigated for hematologic chimerism by PowerPlex System directed to eradicate the malignant clone, such as ASCT. Droplet Digital PCR (ddPCR) is a quantitative approach for the detection of rare allele characterized by a high level of sensitivity and specificity. To evaluate the efficacy of ddPCR JAK2V617F mutation detection assay in monitoring the MRD level at consecutive time-points in a small cohort patients who underwent an ASCT for MF or MF-derived Acute Myeloid Leukemia (s-AML).

Aims: To evaluate the efficiency of ddPCR JAK2V617F mutation detection assay in monitoring the MRD level at consecutive time-points in a small cohort patients who underwent an ASCT for MF or MF-derived Acute Myeloid Leukemia (s-AML).

Methods: DNA from 9 patients affected by primary, secondary MF or s-AML were serially collected during the follow-up after ASCT (50-2500 days). These samples were investigated for hematologic chimerism by PowerPlex System (Promega, USA) and were also analyzed for JAK2V617F mutation both by conventional allele specific PCR (ASO-PCR) and by a validated ddPCR mutation detection assay (Bio-rad, USA). Results were expressed as percentage of JAK2V617F mutated alleles on total evaluated alleles.

Results: The JAK2V617F ddPCR mutation assay was able to detect low muta-
tion load (up to 0.006%), confirming to be much more sensitive than ASO-PCR (0.5-2%). In 4 patients, early after transplantation, we observed by ddPCR a low level of MRD that progressively increased during the follow-up and antici-
pated a decrease in donor chimerism level and a worsening of clinical situation. In 2 patients, who always showed a full donor chimerism and complete hema-
ologic remission of the disease, with very low levels of MRD (from 1% to 0.006%) could be detected by ddPCR in the 2 years after ASCT. With a longer follow-up, a full molecular remission was achieved as demonstrated by ddPCR. In 2 other patients, we observed a very early achievement of full donor chimerism and JAK2V617F molecular negativity (within 90 days post HSCT), also when evaluated by ddPCR. These patients entered a complete hematologic remission of the disease which still persists (after 1 and 5 years after transplantation, respectively). Interestingly, in one patient whose post-transplant hematopoiesis proved full donor and negative for JAK2V617F mutation for 2 years, a weak positive signal revealed by ddPCR (0.075%) became apparent also when evaluated by ddPCR. These patients underwent a hematologic relapse (stable and bone marrow) in subsequent second allogeneic transplant from the same sibling donor restored clinical and molecular remission.

Summary/Conclusions: The ddPCR proved to be a sensitive and accurate method in detecting JAK2V617F mutation. Therefore, this assay can be a valid tool for MRD monitoring in MF patients transplanted without ATG. However, the use of this highly sensitive PCR should be considered with caution in the clinical management of transplanted patients to avoid inappropriate use of donor leukocyte infusion (DLI) and tampering of immunosuppression. A large

Figure 1.
number of patients have to be studied with ddPCR to better understand the clinical significance of low mutation load.

**E1316**

**S100A8/9 ACTIVATION OF MAPK PATHWAY IS SUPPORTED BY ITS RECEPTORS RAGE AND TLR4 IN POLYCYTHEMA VERA**

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**Background:** S100 proteins have been shown to regulate cell proliferation, excessively augmented in myeloproliferative neoplasms (MPN). S100A8/9 is produced by cells of myeloid origin as mediator of inflammation, while AKT and MAPK pathways mediate cell proliferation.

**Aims:** This study analyzed activation of AKT and MAPK pathways by S100A8/9 proteins in healthy controls and MPNs: polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), according to JAK2V617F and calreticulin (CALR) mutation status.

**Methods:** S100A8/9 factor is examined in granulocytes of MPN using immunoblotting, while its influence on cell cycle of granulocytes is determined by flow cytometry. Mutations of JAK2V617F and CALR exon 9 are analyzed by DNA sequencing. Besides JAK2V617F+/ PV patients, we formed per three groups of patients: JAK2V617F+/ CALR+/-, and JAK2V617F- /CALR- for ET and PMF.

**Results:** S100A8/9/Anteilones demonstrated a common significant increase in plasma of MPN patients, whereas the presence of CALR mutation augmented S100A8/9 levels in granulocytes of ET and PMF patients. Activation of AKT pathway is generally reduced by S100A8/9 factor, further on ameliorated by inhibition of the receptor for advanced glycation end products (RAGE) in granulocytes of JAK2V617F+ and JAK2V617F-/CALR+ groups of ET and PMF patients, while it has been prevented by Toll-like receptor 4 (TLR4) inhibition in PV patients. MAPK pathway is significantly inhibited by S100A8/9 only in JAK2V617F+/ ET patients and JAK2V617F-/CALR- PMF patients, partially prevented by TLR4 inhibition in PMF. Inhibition of TLR4 reduced S100A8/9 mediated MAPK activation has been significantly augmented by TLR4 and RAGE inhibition in PV patients. S100A8/9 stimulated granulocyte cycle arrest in G2M phase has been stopped by JAK1/2 inhibition.

**Summary/Conclusions:** S100A8/9 protein levels demonstrated stable elevation in MPN patients. Inhibition of AKT controlled by TLR4, whereas MAPK pathway activation by TLR4 and RAGE in PV, during treatment with S100A8/9.

**E1317**

**MUTATIONAL PROFILE STUDY OF DOUBLE-NEGATIVE ESSENTIAL THROMBOCYTHEMIA BY HIGH-DEPTH NEXT GENERATION SEQUENCING (NGS)**

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**Background:** Essential thrombocythemia is one of the three classical philadelphia negative myeloproliferative neoplasms. It is frequently difficult to diagnose and some molecular markers are used as diagnostic criteria according to WHO classification. Despite this, a significant proportion of patients do not present a clonality marker.

**Aims:** To identify the mutational profile of ET negative for V617F and CALR mutations and to correlate it with clinical data.

**Methods:** A cohort of 22 ET negative for mutations in JAK2 (pQCR) and CALR (GENESCAN) was selected. Median age at diagnosis was 46 years (range: 14-88), male:female ratio 9:13; 2 patients had a record of thrombotic event prior to diagnosis, 4 patients had symptoms at the time of among diagnosis, 3 patients suffered thrombotic event after diagnosis, 1 patient suffered transformation to AML. Median Hb, WBC and platelets at diagnosis were respectively 14.75g/dl, 8.5x1012/L and 720x1012/L. We performed targeted gene sequencing by NGS (Ion Torrent Proton System–Life Technologies) using a panel of 33 genes implicated in leukemia prognosis. X2 and I-student tests were used to find association between mutations and clinical data.

**Results:** On average, 97.94% of the target sequence showed a mean depth coverage around 2500. We discovered 17 non-synonymous mutations which 16 were somatic single nucleotide variants (SNVs) and 1 a nucleotide deletion in coding regions. No mutations were detected in 9 samples (40.9%), 10 samples harbored mutations in 1 gene (45.5%), and the other 3 samples presented 2 or more mutations (13.6%). TET2 was the most frequently mutated gene (18.2%). Other mutations of high prevalence were TET2 (13.6% V617F at a low mean allele frequency (5.8%), MPL (9.1%, one W515L, one with two mutations W515R and S505C, mean allele frequency of 21.95%), SF3B1 (4.5%, one DNM3A (4.5%), and KMT2A (4.5%). The samples with more than one mutation: one presented a CBL and two TET2 mutations, one two mutations in MPL and the other one mutation in TET2 and other in JAK2. No correlation was found between mutational profile and clinical data.

**Summary/Conclusions:** In ET, around 60% of patients present the JAK2V617F mutation, 15-30% show CALR mutations and around 5% present MPL mutations. In spite of this, there is still a significant percentage of ET patients without a molecular marker. Our study shows that the use of a NGS panel allows identifying markers of clonality as for example TET2. NGS also makes affordable to interrogate whole genes classically associated to ET, to detect mutations that were not found by traditional approaches. Finally, we can conclude, as previously described, that ET is an entity with a low mutational burden in comparison with other MPNs as primary myelofibrosis.

**E1318**

**TGR GAMMA CLONALITY ASSESSED BY NGS DOES NOT HELP TO DISTINGUISH EGPA FROM HES**

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**Background:** Hypersensitivity-related syndromes are a heterogeneous group of diseases characterized by sustained and elevated blood eosinophilia with evidence of eosinophil-induced organ damage. Classically, Eosinophilic Granulomatosis with Polyangiitis (EGPA) and Hypereosinophilic Syndrome (HES) represent several overlapping clinical and laboratory features, making it challenging to correctly insert patients in restricted and well-defined categories with specific and more effective therapeutic approaches in daily practice. Therefore, great efforts are ongoing searching for novel biomarkers able to differentiate these two disorders.

**Aims:** To detect T cell receptor gamma (TCRG) clonal rearrangements in EGPA and HES, comparing the frequency of distribution of the V and J region segments in 21 patients afferent to the hematology, rheumatology or pulmonology divisions.

**Methods:** Consecutive patients with a diagnosis of EGPA and HES were enrolled into the study. Inclusion criteria were: documentation of a persistent positive peripheral eosinophilia for at least 3 months. Immunoglobulin, B and T lymphocyte subsets, peripheral blood eosinophilia count of ≥1.5 x 10⁹/L and signs or symptoms of organ involvement. Clinical and laboratory data of the patients were collected. Sequence-based determination of the frequency distribution of TCRG Gene Rearrangements was performed using next-generation sequencing with the Illumina MiSeq (LymphoTrack TCR assay, Invivoscribe).

**Results:** We included 21 patients (9 with EGPA and 12 with HES). Four EGPA patients were MPO-ANCA positive. We detected TCRG clonal rearrangements in 44% patients with EGPA and in 42% patients with HES. No association was observed between TCRG clonal rearrangements and ANCA status in EGPA patients. Following recurrent TCRG gene rearrangements were observed: Vg10JgP1 (5 cases) and Vg4Jg1/2 (4 cases) were observed in both EGPA and HES, whereas Vg9Jg1/2 (2 cases) and Vg10Jg1/2 (2 cases) were observed only in patients with HES. The presence of TCRG rearrangement was not different according to the symptoms (asthma, vasculitis, skin, heart, gut, lung involvement, splenomegaly). IL2, IL5, IL4, eosinophil cationic protein (ECP), absolute eosinophils were measured: ILS and ECP were higher in the polyclonal than in the clonal cases (9.7±2.5 vs 1.7±0.9; p=0.021 and 121.8±61.5 vs 39.5±1.5; p=0.07). On the contrary, no difference was observed in the absolute eosinophil count. Finally, the presence/absence of TCRG clonality did not significantly impact response to treatment (immunosuppressive or interferon) and on the progression-free survival length.

**Summary/Conclusions:** Conclusions: Even if preliminary, this study reveals a similar T cell receptor gamma repertoire in EGPA and HES, with recurrent rearrangements, thus suggesting a possible antigen-driven inflammatory process. T cell receptor rearrangements in both EGPA and HES. Interestingly, this study confirms our previous results showing the TCR delta rearrangement (assessed by qualitative PCR) in 40% of the EGPA patients.
Background: We already demonstrated augmented proinflammatory IL-6 and angiogenic vascular endothelial growth factor (VEGF), hypoxia inducible factor-1α (HIF-1α) and endothelial nitric oxide synthase (eNOS) levels in myeloproliferative neoplasms (MPN).

Aims: To observe IL-6 activated signaling pathways during stimulation of angiogenic factors and their JAK-STAT dependence in MPN.

Methods: We analyzed phosphorylation of JAK/STAT3, PI3K/AKT and MAPK signaling by immunoblotting in HEL 92.1.7 cells (with JAK2V617F mutation) and granulocytes of MPN. The granulocyte cycle phases have been studied by flow cytometry.

Results: We demonstrated IL-6 stimulated angiogenic factors in HEL cells and HEL-derived macrophages, blocked by JAK-STAT inhibition for eNOS and HIF-1α. IL-6 stimulated JAK-STAT3 and angiogenesis related PI3-AKT signaling pathways in HEL cells, the later one prevented by JAK1/2 inhibition. Opposite to primary myelofibrosis (PMF), IL-6 activation of JAK-STAT3 and PI3-AKT pathways has been prevented and enhanced by JAK1/2 inhibition, respectively in granulocytes of polycythemia vera (PV). Moreover, IL-6 inhibition of JAK-STAT3 and PI3-AKT pathways in essential thrombocythemia (ET) has been prevented by JAK2 inhibitor in JAK2V617F positive ET granulocytes. JAK1/2 inhibitor Ruxolitinib upregulated IL-6 activates of MAPK pathway in MPN, in contrast to specific JAK2 inhibitor Hexabromocyclohexane. IL-6 mediated reduction in the percentage of HEL cells in G2M phase was reversed by Ruxolitinib that potentiated apoptosis and reduced the cell percentage in G0-G1 phase both in HEL cells and granulocytes of PMF. It has been detected the cell cycle arrest of MPN granulocytes in S phase (DNA replication) after treatment with IL6, completely diminished by JAK1/2 inhibition.

Summary/Conclusions: Therefore, we concomitantly revealed that inflammation stimulated angiogenic factors and signaling pathways involved in cell proliferation, apoptosis and angiogenesis are regulated by JAK-STAT inhibition.

Myeloproliferative neoplasms - Clinical

E1320

PERCEPTION OF SYMPTOM BURDEN AND TREATMENT GOALS BETWEEN PHYSICIANS AND PATIENTS WITH MPNS: AN ANALYSIS FROM THE INTERNATIONAL MPN LANDMARK SURVEY

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Background: The global MPN LANDMARK survey evaluated the patient (pt) and physician-reported impact of myeloproliferative neoplasms (MPNs), including myelofibrosis (MF), polycythemia vera (PV), and essential thrombocythemia (ET), among pts from 6 countries. We present an analysis comparing physician and pt perceptions of the impact of these MPNs.

Aims: To investigate differences between pt and physician perceptions of symptom burden, treatment goals, and disease management.

Methods: This was a cross-sectional survey of pts with MPNs and physicians treating pts with MPNs. Respondents completed an online survey ranking their perception of the impact of MPNs on symptom burden, disease management, and treatment goals. Pts and physicians were recruited independently.

Results: Pts (n=699) from Australia (n=10), Canada (n=64), Germany (n=149), Italy (n=106), Japan (n=84), and the UK (n=286) completed the survey (MF, n=223; PV, n=174; ET, n=302). Most pts had been diagnosed within ≤2 years of experiencing symptoms (73%); 56% were women. Physicians (n=219) were from the same countries; most were hematologists (54%) or hemato-oncologists (27%). Overall, 54% of pts reported having a prognostic score; however, 71% of physicians reported using a prognostic risk classification. Physicians assessed symptoms by proactively asking pts how they were feeling (43%) or asking about specific symptoms (37%); 11% waited for pts to mention symptoms. Importantly, only 26% of physicians used a validated symptom assessment form; 44% used their own rating method. Pts and physicians both agreed that pts with MPNs have a high symptom burden and that MF had a higher degree of burden on daily living. Interestingly, a higher proportion of physicians...
than pts felt that MPN symptoms have an impact on pt quality of life (92% vs 76%) and that pts had a substantial emotional burden associated with their disease. For instance, 34%, 29%, and 26% of pts with MF, PV, or ET reported feeling anxious or worried compared with 70%, 46%, and 36% of physicians reporting that their pts experience substantial anxiety or worry. Some pts did not recognize that their symptoms could be MPN related; for example, = one-fifth of pts did not think that their night sweats could result from their MPN (16% MF, 21% PV, 25% ET). Consistent with this, 60% of physicians indicated that pts could identify only few or some of their symptoms as MPN related. Pts and physicians were both concerned about reducing symptoms (pts: 70% MF, 61% PV, 53% ET; physicians: 80% MF, 55% PV, 60% ET); however, pts were also concerned about delaying MPN progression (58% MF, 57% PV, 66% ET; physicians: 43% MF, 28% PV, 37% ET; Figure 1). Compared with pts, physicians indicated a greater focus on prevention of vascular/thrombotic events in PV (66% vs 48%) and ET (80% vs 60%). Overall, only 27% of physicians felt they completely agreed with their pts on treatment goals; 66% felt they “somewhat” agreed. However, most pts (87%) were satisfied with their physician’s disease management/communication.

Summary/Conclusions: This study revealed a potential disconnect between physician and pt perceptions relating to communication and disease management, and an apparent lack of standardization in symptom assessment. Of note, some pts did not recognize that their symptoms could be MPN related and had different treatment goals than their physicians, indicating a need for improved pt education and pt-physician communication and a treatment plan that includes standardized monitoring of symptoms and agreement on treatment goals.

E1321

BASELINE QUALITY OF LIFE INDEPENDENTLY PREDICTS OVERALL SURVIVAL IN THE MYELOFIBROSIS: KEY INSIGHTS FROM THE COMFORT-I STUDY

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Aims: To evaluate the prognostic relevance of QOL and symptom burden among patients with MF from the COMFORT-I study.

Methods: Data from the COMFORT-I trial of ruxolitinib (Verstovsek 2012) versus placebo was obtained from Incyte® for independent analysis. Association of total symptom burden (TSS; divided by the sample quartiles) and QOL (divided by the sample median) at baseline with OS among MF patients was estimated using the Kaplan-Meier method and tested using log rank tests and Cox regression. Symptom burden and QOL were assessed using the 5-symptom MF-SAE high risk (Mesa 2009) and FORTC QOL-C Global Health/QOL scale (Aaronson 1993), respectively. The PROMIS instrument was used to assess fatigue (Cella 2007).

Results: A total of 309 patients were available for analysis including 155 ruxolitinib-treated and 154 placebo-treated MF patients. Baseline demographics, disease-related variables, and calculated overall survival were similar to previous published results (Verstovsek 2015). Symptom Burden: When comparing OS by TSS quartiles at baseline, no significant associations in OS were observed (Figure 1A). Individual symptoms of bone or muscle pain, feeling full, pain under ribs on left side, abdominal discomfort, itchiness, or night sweats did not demonstrate significant associations when comparing OS by quartile symptom score. Baseline fatigue score demonstrated no difference in OS when stratified by median or quartiles. Global Health Status/QOL: Intention to treat analysis demonstrated significant survival advantage for patients with higher QOL at baseline (HR 1.47, p=0.02, Figure 1B). When censoring placebo patients at crossover, this hazard ratio improved to a HR 1.79 (p=0.008). Cox Proportional Hazards Modeling: Cox regression for survival analysis reached significance for items of age (p<0.001), sex (p<0.009), and QOL (p=0.009) when taking into consideration TSS, IPSS prognostic risk score, age, sex, COMFORT treatment arm, and QOL. When censoring for placebo patients at crossover, this analysis demonstrated that the same items remained significant (age [p<0.001], sex [p<0.001], and QOL [p=0.002]).

Summary/Conclusions: For the patients prospectively evaluated in the COMFORT-I trial, pre-treatment QOL is strongly prognostic for overall survival and represents a strong independent factor associated with survival. Prior literature has confirmed the importance of QOL in prognosticating survival in other cancer types. However, this is the first study that has identified the key correlation among individuals with MF. Neither individual nor combined symptom scores at baseline appeared prognostic for overall survival, emphasizing the importance of QOL assessment in addition to symptom assessment. Weight loss (a prognostic factor for DIPSS scoring) was not included in this symptom burden assessment and may represent an independent factor associated with increased survival.

E1322

CHARACTERIZATION OF DISEASE AND OUTCOMES OF PATIENTS WITH MYELOFIBROSIS: A POPULATION BASED STUDY

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Aims: This population-based study characterizes disease and outcomes in patients (pts) with MF by using the U.S. Surveillance, Epidemiology, and End Results (SEER) database.

Methods: We identified a total of 3,367 pts with primary myeloid fibrosis (PMF, ICD-O-3 morphology code as 9961/3 and primary site code as C420, C421 or C424) diagnosed between January 2000 to December 2013. Pts with missing survival status (n=753), pts lost to follow up (n=4), and pts with missing age record (n=1) were excluded. Kaplan-Meier analysis was performed to determine overall survival (OS) and cancer specific mortality. The effects of specific covariates on OS were analyzed using a Cox proportional hazards model.
Results: The final study cohort comprised of 2,619 PMF pts. Median follow up period was 28 months (interquartile range 95-77 years) with 60.6% (n = 1,586) ≥ 65 years old. More than half of the pts were male (58.5%; n=1,531); 82.2% (n=2,153) were white, and 16.4% (n=430) were diagnosed between 2012 and 2013. The geographic distribution was as follows: East 14.8%, South 18.4%, West 54.2% and Midwest 12.6%. Median OS was 42 months (Figure 1). The hazard ratio of all-cause mortality for age was 1.05 (95% Confidence interval (CI) 1.04-1.05), for female vs male was 0.72 (CI 0.64-0.80), for nonwhite vs white 1.01 (CI 0.87-1.16), for unmarried vs married was 1.04 (CI 0.94-1.16), for patients diagnosed 2012-2013 vs 2000-2011 was 0.95 (CI 0.75-1.20). Compared to West, the hazard ratio of OS for East, South and Midwest was 1.05 (CI 0.90-1.22), 1.28 (CI 1.12-1.47), 1.03 (CI 0.88-1.19) respectively.

Summary/Conclusions: This population based study showed that the overall survival of pts with PMF was short. Older and male pts were associated with higher mortality risk. There were significant differences across geographic regions of the United States. Although there is a trend of improvement in the period of 2012 to 2013, the result is not statistically significant, partially due to short follow up. These findings underscore the continuing need for effective therapies for pts with MF.

E1323

SERUM ALBUMIN IS A STRONG PREDICTOR OF SURVIVAL IN MYELOFIBROSIS, INDEPENDENT OF IPSS, DIPSS, AND DIPSS+ SCORES

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Background: Albumin is the main protein in human plasma. Serum albumin (SA) is used as a surrogate marker of nutritional status and inflammation. The prognostic role of SA has been studied in many diseases, including hematologic malignancies. In myelofibrosis (MF), ruxolitinib has been shown to improve SA levels in addition to other metabolic parameters. SA holds particular significance in MF given its ability to capture both nutritional status and inflammation level in a disease hallmarked by hyperactive inflammatory pathways and constitutional symptoms.

Aims: We aim to closely evaluate the significance of SA in MF patients as it pertains to clinical presentation, laboratory correlations, disease genomics, comorbidities and outcomes.

Methods: We retrospectively reviewed an institutional database of 376 MF patients who presented to Moffitt Cancer Center between 1/1/1998 and 12/31/2012 and had available SA levels within 30 days of presentation. Laboratory values and prognostic scores were determined at time of first presentation. Overall survival (OS) was measured from time of first presentation until date of death or censored at time of last follow-up. Progression free survival (PFS) was defined as time from first presentation to development of acute myeloid leukemia (AML).

Figure 1.

Results: Our cohort of MF patients had median age of 67 and 69 at diagnosis and presentation, respectively. Most patients had primary MF (73%) with 11% and 16% having post-PV MF and post-ET MF, respectively. First, we looked at the correlation between SA and other clinical factors. SA was positively correlated with hemoglobin (p<0.01) and platelet count (p<0.01), and negatively correlated with age (p<0.01), peripheral blast percentage (p=0.03), ferritin (p<0.01), prognostic scoring models (p<0.01 for IPSS, DIPPS and DIPSS+) and pack-year smoking history (p<0.01). SA did not correlate with spleen size or any specific somatic mutation, but negatively correlated with somatic mutation burden (p=0.03). On univariate regression, SA was associated with inferior PFS (HR: 0.31 [0.13-0.72]; p<0.01) and OS (HR: 0.25 [0.17-0.36]; p<0.01). Four cohorts were created based on SA: cohort I: SA 2.5-3.5 g/dL (n=31); cohort II: SA 3.6-4.0 g/dL (n=98); cohort III: SA 4.1-4.5 g/dL (n=182); and cohort IV: SA >4.5 g/dL (n=84). OS increased with increasing SA; with median OS (in months) of 9.34, 25.3, 48.4, and 84, defined in cohorts I-IV, respectively. On focused comparison, each cohort was significantly different than all others. On multivariate analysis, the influence of SA on OS remained significant after controlling for prognostic scorings (IPSS, DIPPS, DIPSS+) and comorbidities. For PFS, SA remained significant when controlling for IPSS and DIPPS (p<0.01) but lost significance (p=0.08) when controlling for DIPSS+. Multivariate analysis was performed on a cohort of patients with available molecular data (n=138). SA significantly influenced OS after controlling for prognostic systems, comorbidities and mutations of SRSF2 and ASXL1. Lastly, given its independent prognostic influence and incorporation in more complex scoring systems, SA was evaluated in a multivariate model for OS in terms of its ability to improve prediction accuracy. The incorporation into known prognostic scoring systems provides an improved ability to accurately capture low and high-risk subgroups.

Summary/Conclusions: SA level is independently prognostic in MF and correlates with variables known to hold prognostic value. Its representation of nutritional status, inflammation, and comorbidities imbues it with special status in predicting outcome. Its incorporation into known prognostic scoring systems provides an improved ability to accurately capture low and high-risk subgroups.

E1324

CLINICAL UTILITY OF NEXT-GENERATION SEQUENCING IN THE MANAGEMENT OF MYELOPROLIFERATIVE NEOPLASMS

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Background: Although Next Generation Sequencing (NGS) has helped characterize the complex genomic landscape of myeloid malignancies, its clinical utility remains not well defined. Funding for NGS testing by healthcare systems or third party payers is variable due to the lack of data on its utility in a routine care setting. At our centre, targeted sequencing (TAR-seq) is offered to all new patients referred for myeloid malignancies as part of the Advanced Genomics in Leukemia (AGILE) program.

Aims: In this study, we evaluate the impact of TAR-seq on the management of patients with a diagnosis of MPN or post-MPN acute myeloid leukaemia (MPN/AML).

Methods: All consenting patients referred to the MPN program at the Princess Margaret Cancer Centre between February 15 and December 16 with a suspected or confirmed diagnosis of MPN were evaluated (n=188). TAR-seq was performed on DNA extracted from peripheral blood (n=159, 85%) or bone marrow (n=29, 15%) using the TruSight Myeloid Sequencing Panel (Illumina), a targeted NGS panel of 54 genes (39 hotspot region; 15 complete coding region coverage) implicated in myeloid malignancies. Reporting was performed by high quality exonic nonsynonymous, intronic splice site, frameshift, nonsense and known pathogenic synonymous variants. Variants with global mean allele frequency >1% were identified using multiple population databases (1000 genomes, ESP, ExAC) and excluded. Each patient’s TAR-seq results were reviewed alongside their clinical information systematically by at least two hematologists with expertise in MPN, and disagreements were resolved by consensus.

Results: 170 patients fulfilled the 2008 WHO diagnostic criteria for MPN: 107 were diagnosed with myelofibrosis (MF), 26 with polycythaemia vera (PV), 21 with essential thrombocythaemia (ET), 11 with other MPN, and 12 were unclassifiable with 12 and with MPN/AML. In 6 patients with ‘triple negative’ MPN, who lacked mutations in the driver genes JAK2, CALR and MPL, TAR-seq confirmed clonal hematopoiesis through identifying other mutations. In 61 transplant-eligible patients with MF, 32 (52%) were considered to carry a high molecular load (ML) profile based on mutational load at an ML cut off in A S RS F 2, EZH2, IDH1/2, SRSF2 or TP53 or a total of three or more mutations. Of these, 11 patients (34%) were considered for early transplant, three with Intermediate-1 and eight with Intermediate-2 risk, who were responding well to JAK 12
inhibitor (JAKi) therapy. All high-risk, transplant-eligible MF patients were con-
sidered for transplantation irrespective of their HMR status. Nine patients with
low/intermediate-1 risk MF bearing HMR mutations were considered for a clin-
tical trial of early JAKi therapy, and one patient was successfully enrolled. Seven
patients were identified with IDH1/2 mutations (five with MF and two with
MPN/AML), and therefore can be potential candidates for enrolment into clinical
trials of JAK inhibitors. In PV and ET, TAR-seq identified HMR profiles in 6/26 (23%)
and 5/21 (24%) patients, respectively. These
patients are monitored closely, but no therapeutic decisions were taken based
on their HMR profile. In MPN/AML, TP53 mutations were detected in 4/12
(33%) patients. However, these patients progressed rapidly before their TAR-
seq results became available to inform clinical management.

Summary/Conclusions: We have determined that TAR-seq improves the
correlation of tissue-negative MPN patients, refines risk stratification and
decisions related to the timing of transplant in MF, and can potentially identify
candidates for future targeted therapies. Therefore, we suggest that NGS shall
become part of the standard of care in MF, be part of the investigation of
multiple negative MPN. Based on these findings and in conjunction with ongoing studies
in the MPN program, an algorithm integrating NGs in the management of MF
has been developed, and will be evaluated prospectively.

E1325
IMPACT OF COMORBIDITIES AND BODY MASS INDEX ON SURVIVAL IN
PATIENTS WITH MYELOFIBROSIS TREATED WITH RUXOLITINIB
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Background: Charlson Comorbidity Index (CCI) and body mass index (BMI)
are significantly associated with outcome in patients (pts) who receive continue treatment with
tyrosine kinase inhibitors. Ruxolitinib (RUX) is the first JAK1/2
inhibitor that may induce spleen/symptom responses and improve quality of
life in pts with myelofibrosis (MF). No data are yet available on the impact of
comorbidities and BMI on pts treated with RUX.

Aims: To evaluate the impact of CCI and BMI on overall survival (OS) in
a cohort of RUX-treated MF pts.

Methods: A multicenter observational study on WHO-defined MF treated with
RUX according to standard clinical practice was conducted in 20 Italian Hema-
tology Centers. Response to RUX was evaluated according to 2013 IWG-MRT
criteria. OS was calculated from the date of RUX start to the time of death or
last follow-up. Baseline parameters evaluated for correlation with OS were:
blood count, spleen ≥10cm, marrow fibrosis grading, time from MF diag-
nosis to RUX start, transfusion dependency, mutation status, Total Symptom
Score (TSS), CCI, and BMI.

Results: Between June 2011 and Apr 2016, 343 pts with PMF (51.9%), or
post-ET (20.1%) / post-PV (28.0%) were treated with RUX in participating Cen-
ters. At RUX start, median age was 67.6 years (range 36.5-89.0) with a male
prevalence (57.1%); International Prognostic Score System (IPSS) was inter-
medial in 115 (41.8%) pts, high in 51 (15.5%) and very high in 36.4%.
Transfusion dependency and spleen enlargement were present in 23.9% and
97.4% of pts, respectively (62.4% with spleen ≥10 cm). TSS was <20 in 131 pts
(38.2%); 62 (18.1%) pts had a BMI<21 (corresponding to lower quartile). CCI was
zero in 105 pts (30.6%), one in 74 pts (21.6%), two in 58 pts (16.9%) and ≥3 in 106 pts
(30.8%). The achievement of a spleen response at 6 months significantly increased OS (Fig. 1A). Also, a higher CCI
did not correlate with lower spleen response at 6 months (44% vs 34% of pts
with CCI<3, p=0.11). The impact of higher CCI on survival was only mildly
affected by the achievement of a spleen response at 6 months (Fig. 1B).

Figure 1.

Summary/Conclusions: Together with transfusion requirement, CCI and BMI
can influence survival in RUX-treated MF pts. Taking into account these addi-
tional parameters may allow to better define survival probability beyond IPSS
risk assessment. Unfavorable CCI and BMI did not hamper responses to RUX;
also, the achievement of a spleen response counterbalanced the negative
prognostic effects of a lower BMI.

E1326
ANALYSES OF 845 PATIENTS WITH PMF, PET-MF AND PPV-MF TREATED
IN 35 GERMAN HEMATOLOGY CENTERS – A RETROSPECTIVE FIELD
STUDY
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Background: Primary myelofibrosis (PMF) as well as secondary post essential
thrombocythemia (peT-PMF) and post polycythemia vera (pPV-PMF) are consid-
ered rare diseases associated with significant morbidity. Diagnostics and ther-
apeutic options have significantly improved during the last decade by develop-
ment of novel drugs, improvement of allogeneic stem cell transplantation (SCT)
procedures and supportive care. Whereas the characteristics of PMF, peT-PMF
and pPV-PMF patients (pts) participating in clinical trials are well analyzed, data
are rare for the general MF population including patients not included in or eli-
gible for clinical trials.

Aims: In order to gain a broader, more comprehensive data set on the general
MF population we performed a questionnaire poll in 35 German hematology
centers gathering characteristics on 845 pts who were currently under care.

Methods: A questionnaire asking for general patient and disease specific data
as symptoms, spleenomegaly, prognostic factors, past/current treatment and
blood count, degree of MF in bone marrow and transfusion frequency was
designed. It was distributed to participating centers (n=35, mostly private
offices) throughout Germany and analyzed centrally. Time period of collection

Figure 1.
was 03/2013-12/2015. 845 pts were included i.e. a median of 20 pts (range 6–90 pts) per center

**Results:** Gender was equally distributed (50%/50%). Pts ages at initial diagnosis were as follows:<50 years (11%), 50–69y (19%), 70-79y (31%), and >70y (40%). Current age was >65y in 70% of all pts. PMF represented the largest MF cohort (77%), followed by pET-MF (10%), pPV-MF (7%) and unspecified (6%). Most pts achieved disease control (12-month duration >50%), <1y (15%); unknown (1%). Key current blood values at time of discovery included abnormal thrombocyte counts (<500GPT/l; 50 <1000GPT/l (10%); ≤450GPT/l (28%) and elevated WBC >25.000/μl (11%). Presence of circulating blasts in the peripheral blood was documented in 11% of pts. Hemoglobin [g/dl] was ≥10 (68%), <8 (10%), and unknown for 3% of the pts. 26% of the pts had a history of treatment before diagnosis, with 24% of the pts currently treated according to local protocols. We collected clinical data of patients at diagnosis, and repeated 6-9 months after the start of therapy (for pts that progressed as in the trials. Interestingly gender was equally distributed in our study. SCT was a rarely used treatment within this cohort whereas JAK2 inhibitors were frequently used

**E1327**

**CALR MUTATION TYPE INFLUENCES THE RISK OF THROMBOSIS IN ESSENTIAL THROMBOCYTEMA ACCORDING TO A COOPERATIVE STUDY BETWEEN TWO SPANISH CENTERS**

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**Background:** Driver mutations in ET include JAK2V617F, CALR and MPL genes. The mutation profile affects the hematological parameters and the risk of thrombosis being the JAK2V617F mutation the one associated with the higher risk of thrombosis. Among CALR mutations there are two main types: type-1 like and type-2 like, but it is not clear if the mutation type is associated with a different clinical feature

**Aims:** The objective of this study is to determine the clinical meaning of CALR mutation type in ET

**Methods:** We analyzed 309 ET patients from two hospitals: H.C.U. Santiago and U. of Gran Canaria Dr. Negrin. Dates of diagnosis were between 1-11-2010 and 1-1-2016, and the median follow up was of 6.88 years. Patients were treated according to local protocols. We collected clinical data of patients at diagnosis and during follow-up as well as events such as thrombosis, transformation to myelofibrosis (MF) or acute leukemia (AL). Thrombosis associated with diagnosis refers to those events happening from two years before diagnosis until diagnosis. The statistical analyses were performed with R Core Team (2016) and IBM SPSS 21.0

**Results:** JAK2V617F mutation was present in 60.5% of the patients, 1.9% had MPL mutations, 14.5% were CALR type-1like, 11% were CALR type-2like and 11% were without mutation. In three cases, we were not able to classify CALR mutation as type-1/2 like. With regard to the clinical events: 21 patients (6.8%) had thrombosis associated with diagnosis, and 34 (11%) at least 1 thrombosis since the diagnosis. Twelve patients suffered more than 1 thrombotic event. MF evolution was found in 18 patients (5.6%) and 2 cases transformed to AL. In 31% of pts, thrombocytosis >600GPT/l was observed during the course of the disease. Thrombocyte counts (mean 3.8x109 vs 8.9x109/L, p<0.001) and also in JAK2V617F vs. CALR type-2like (p=0.013). When comparing CALR type-1like vs CALR type-2like the differences were marginally significant (p=0.06). In a multivariate analysis with the IPSET variables and CALR subtype, in our series the previous history of thrombosis (p <0.001) and the JAK2V617F status (p=0.026) were significantly associated with increased risk of thrombosis, but no the advanced age neither the presence of cardiovascular risk factors. However, presence of CALR type-2like mutation, with respect to the JAK2V617F mutation, was a protective factor of thrombosis (p=0.06). The five year-thrombosis free survival (TFS) study was as follows: 83%, 85% and 97% for groups JAK2V617F, CALR type-1like and CALR type-2like (log rank p=0.03)(fig. 1).

**Summary/Conclusions:** The type of driver mutation is associated with a different risk of thrombosis. Among the two types of CALR mutation, patients have similar clinical characteristics except for the risk of thrombosis which seems lower in CALR type-2like compared to type-1like. This finding shows the importance of studying the CALR mutation type in ET.

**E1328**

**MONITORING OF LEUKOCYTE-PLATELET AGGREGATES AND SELECTIN LEVELS IN PATIENTS WITH PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS**

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**Background:** Although the reduction of thrombotic risk is a primary goal of therapy in Philadelphia negative myeloproliferative neoplasms (Ph-MPN), even low risk patients (pts) may experience thrombotic events during the course of the disease. Some recent studies revealed a correlation between the occurrence of thrombosis and activation of blood and endothelial cells. However, not many information is available about influence of therapy on these parameters

**Aims:** We prospectively analyzed the levels of leukocyte-platelet (Lept) aggre-
gates, together with levels of soluble selectins, in a group of pts with Ph MPN with diagnosis and during therapy

**Methods:** Our study included 80 consecutive de novo Ph MPN pts (37 poly-
cythemia vera, 27 essential thrombocythemia, 26 primary myelofibrosis), diagnosed according to WHO criteria. According to therapy, pts were assigned as: hydroxyurea (HU 7.8%), aspirin (ASP 55.6%), hydroxyurea+aspirin (HU+ASP 31.1%), and 5.6% of pts were not on therapy. Neutrophil-platelet (Neu-
PT) and monocyte-platelet (Mo-PT) aggregates were determined in whole blood samples (EDTA/CTAD) by flow cytometry. Aggregates were estimated as fraction (%) of CD42b and CD61* neutrophils and monocytes. Plasma levels of E-, L- and P-selectins were determined by enzyme immunoassay. All analyses were performed on diagnosis and 6 months after the start of therapy (for pts on HU after achievement of partial or complete remission).

**Results:** In all pts, mean levels of Neu-Pt and Mo-Pt aggregates at diagnosis were significantly elevated in comparison to control values (22.9% vs 8.9% and 13.0% vs 5.2% respectively, p<0.01). Mean concentration of soluble E-, L- and P-selectins were also significantly higher in Ph-MPN than in control group (34.2 ng/mL vs 19.0 ng/mL, 2748.7 ng/mL vs 1322.0 ng/mL and 294.0 ng/mL vs 69.8 ng/mL, respectively, p<0.01). Mean levels of Neu-Pt and Mo-Pt aggregates in response to therapy were significantly reduced compared to baseline levels (Figure). Significant reductions were observed for E-selectin levels in HU+ASP group, for L-selectin levels in all three therapy groups and for P-selectin levels in HU and HU+ASP groups (Table). During the median follow up of 39 months from diagnosis of Ph-MPN, thromboembolic events occurred in 13.3% of pts (12/90), particularly: 0/7 on HU, 3/50 on ASP, and 9/28 on HU+ASP. In this subgroup we observed increased baseline levels of Neu-Pt and/or Mo-Pt aggregates in 9/12 pts, while all 12 pts had increased at least one soluble selectin, predominantly P-selectin. Retesting revealed that all 9 pts with thrombosis and increased aggregates level at baseline, normalized those levels after therapy, while only 4/12 pts normalized soluble selectin levels.

**Figure 1.**
HEAT SHOCK PROTEIN 27 EXPRESSION IS INCREASED IN PATIENTS WITH PRIMARY AND SECONDARY MYELOFIBROSIS AND MAY BE AFFECTING THEIR SURVIVAL

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Background: Increased heat shock protein 27 (HSP27/HSPB1) expression and phosphorylation were observed in a large number of neoplastic diseases and they have mostly been associated with aggressive disease features and poor prognosis. There are only few reports investigating HSP27 in primary myelofibrosis (PMF), a myeloproliferative neoplasm characterized by high inflammatory state reflecting in debilitating clinical symptoms.

Aims: To analyze HSPB1 mRNA expression in patients with PMF and secondary myelofibrosis (SMF) and to correlate it with clinical and hematological features.

Methods: We analyzed HSPB1 relative expression in bone marrow aspirates of 26 patients with PMF, four patients with SMF and 13 controls using quantitative real time polymerase chain reaction (RT-PCR). Spleen size was assessed by palpation. Association with overall survival was analyzed in 27 PMF and SMF patients evaluated at the time of diagnosis. The Kuakul-Walls one way analysis of variance, The Mann Whitney U test, the Chi squared test, Spearman rank correlation, the log-rank test and the Cox regression analysis were used, cut-off point for survival analyses was determined using the ROC curve analysis.

Results: Relative expression of HSPB1 differed significantly between diagnoses (P=0.011); it was significantly higher in patients with PMF and SMF than in control group (P<0.05 for both comparisons), but did not differ between PMF and SMF patients (non significant). Increased expression was associated with increase in the spleen size (P=0.009) and JAK2 V617F mutation (P=0.073). We did not detect significant associations with other disease specific features. Lower HSPB1 expression was associated with inferior overall survival in both univariate (HR 3.2; P=0.04) and multivariate analysis (HR 6.12; P=0.004) where effect was independent of age (non significant), gender (non significant) and the International Prognostic Scoring System (IPSS) score (HR 3.31; P=0.033).

Summary/Conclusions: We have found elevated of blood and endothelial cell activation markers at baseline in Ph-MPN. Cytoreductive and antiaggregatory therapy reduced the mean level of Le-Plt aggregates and concentration of soluble selectins. In subset of pts with thrombosis, therapy lead to normalization of Le-Plt aggregate levels, with incompletely normalized soluble selectin levels. Even with normal Le-Plt aggregates, observed elevated selectin levels can explain persistent thrombotic risk due to intrinsic changes in relationship between blood and endothelial cells as a part of biology of Ph-MPN itself.

E1329
NON-DRIVER MUTATIONS IDENTIFIED BY A 190-GENE NEXT GENERATION SEQUENCING PANEL IN PATIENTS WITH PRIMARY MYELOFIBROSIS AND POST-POLYCYTHEMIA/ESSENTIAL THROMBOCYTHEMIA MYELOFIBROSIS

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Background: It is a consensus that the driver mutation is an independent prognostic factor in PMFs. Moreover, some non-driver mutations are found associated with initiation, progression and prognosis in PMFs. However, a recent study from the AGIMM (AIRC- Gruppo Italiano Malattie Mieloproliferative) group showed that the type of driver mutation did not influence prognosis in post-PV/ET MF. These observations proved that there were indeed some differences in these two types of MF.

Aims: The aim of current study was to describe the non-driver mutation landscape and the molecular differences between the patients with PMF and those with post-PV/ET MF.

Methods: Targeted gene sequencing was carried out at diagnosis. We sequenced 190 genes across 62 patients, resulting in 229 high-confidence mutations. The average gene coverage was 99%. The average read depth was 540×. Also, 92% of targeted regions were covered with >20×. Every mutation identified in this study was then compared against these expected patterns and categorized into "oncogenic," "possible oncogenic variants," or "unknown significance". Using copy number-adjusted VAF, we reconstructed the clonal architecture to establish whether a mutant gene was an ancestral or subclonal mutation. According to the statistically differences in VAF among gene mutations, subjects were classified as two different clonal architecture, namely clone+subclone(s) (P<0.05) or clonal.

Results: In PMFs, 42 (93.3%) patients had at least one non-driver mutation. Within the 17 patients lacking the driver mutations in JAK2V617F/Exon 12, MPLW515 and CALR, 2 had mutant genes (SH2B3 and PIAS3) involving in JAK-STAT pathway, 13 had mutations in other genes and 2 had no mutations. In Post-MFs, non-driver mutations were detected in 16 (94.1%) patients. There are no differences in the median number of non-driver mutations in PMFs vs. post-PV/ET MFs (3 vs. 3.18, P=0.885) and PMF patients with vs. without driver mutations (3 vs. 3.18, P=0.668). In PMFs, 12 non-driver genes were mutated in >5% of patients, namely ASXL1 33.3%, U2AF1 22.2%, TET2 15.6%, FAT1 15.6%, SETBP1 13.3%, SRSF2 8.9%, CUX1 8.9%, EP300 8.9%, FAT2 6.7%, NOTCH3 6.7%, EZH2 6.7%, and GATA3 6.7%. In post-PV/ET MFs, ASXL1 (41.2%) was the most frequent mutation, followed by TET2 (29.4%), U2AF1 and SRSF2 mutations were significantly more frequent in PMF than in post-PV/ET MF. Moreover, SETBP1 and FAT1 were mutated in PMF more often and not mutated in post-PV/ET MF. Figure 1 A-C show 3 illustrative patients. Clonal architecture was significantly different between PMFs and post-PV/ET MFs (Figure 1D). About 50% PMF patients were classified as clonal, however, most (87.5%) post-PV/ET MF patients were clone+subclone(s). In PMFs, driver mutation was an ancestral mutation with other non-driver mutations in 14 (31.1%) subjects as 2015-R02413 in Figure 1A. Moreover, driver mutation even was a subclonal mutation in 9 (16.7%) subjects as 2015-R02406 in Figure 1B.
1B. In post-PV/ET MFs, 11 (64.7%) subjects showed that JAK2 mutation as an only ancestral mutation as G1215G00701 in Figure 1c.

Figure 1.

Summary/Conclusions: In conclusion, we found that the differences in non-driver mutation profile and clonal architecture between PMF and post-PV/ET MF. In addition, by applying a 190-gene panel we demonstrated some variants classified as of “unknown significance”. And larger sample sizes may enable some of these to be reclassified in the future. The precise role of each mutation and their impact on MPN phenotype will require further studies.

E1331

DETERMINING MEANINGFUL CHANGE IN THE MYELOFIBROSIS SYMPTOM ASSESSMENT FORM (MFSAF) v2.0 USING A COMBINATION OF DISTRIBUTION- AND ANCHOR-BASED APPROACHES IN THE COMFORT-I TRIAL


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Background: Symptom response was defined in the COMFORT-I trial as a 50% improvement from baseline at week 24 in the Myelofibrosis Symptom Assessment Form (MFSAF) v2.0 total symptom score (TSS; Mesa [J Clin Onc, 2013]; 0 to 60 scale where 60 represents the worse symptom experience imaginable) with no minimum score requirement at baseline.

Aims: In this analysis of the phase III placebo-controlled COMFORT-I study we used distribution- and anchor-based approaches to investigate whether alternative change scores in the MFSAF v2.0 TSS could be meaningful relative to patient-reported quality of life (QOL).

Figure 1.

Methods: One third and one half of the pooled standard deviations (SD) of scores and change scores (raw and percentage change) were used as distribution-based estimates. The anchor-based approach estimated meaningful changes (raw and percentage change) relative to the patient’s change in global health status/QOL (GH/QOL; 0 = worst, 100 = best) as measured by the EORTC QLQ-C30 where a decrease of 12.1 or more points was considered as deterioration; an increase of 7.6 or more points was considered as improvement; and all other changes were considered as stable based on change scores established in a multiple myeloma population (Kvam et al., Eur J Hem, 2011). Analysis of covariance (ANCOVA) was used to investigate whether estimated meaningful changes were consistent across the spectrum of observed baseline TSS. This model of TSS changes at week 24 included a continuous term for baseline TSS, a 3-level grouping factor for GH/QOL change (deterioration vs stable vs improvement), and an interaction term between baseline TSS and the GH/QOL grouping factor.

Results: 301 patients randomized to ruxolitinib [N=149] or placebo [N=152] completed TSS at baseline (45% female, median age 68 [range 40-91]). Median baseline TSS was 16.8 (range 0 to 52.7). Pooled SD at baseline and week 24 in TSS was 11.4 and 11.6, respectively, resulting in estimated meaningful changes of 3.8-5.8 points. For change and percentage change from baseline at week 24 in TSS, the pooled SDs were 9.8 and 75%, respectively, resulting in estimated meaningful changes of 3.3-4.9 points or 25%-38%. Among patients with TSS and QLQ-C30 data at baseline and week 24, 51 (23%) patients had deterioration, 61 (27%) were stable and 110 (50%) had improvement based on QLQ-C30 GH/QOL changes. Mean (95% CI) changes in TSS for the three groups were 0.8 (-2.5 to 4.2), -1.4 (-3.8 to 0.8), and -6.8 (-9.0 to -4.6), and for percent changes 20% (-6% to 46%), 17% (-11% to 44%) and -34% (-45% to -22%). ANCOVA revealed that baseline TSS statistically significantly impacted meaningful change estimates (p < 0.0001). For Figure 1, estimated mean (95% CI) changes in TSS for the improved group of -20.8 (-26.4 to -15.1), -11.7 (-14.3 to -9.0), and -2.6 (-5.1 to -0.1) for baseline TSS of 50, 30, and 10.

Summary/Conclusions:Distribution- and anchor-based approaches suggest that changes as small as 3-6 points on a 0-60 scale of the MFSAF v2.0 TSS may be meaningful to patients. However, estimates of meaningful change appear to increase in magnitude for higher baseline scores, though in a way that a static percentage change criterion would either require too much change for lower baseline TSS or not enough change for higher baseline TSS. All analyses suggest that some changes in symptoms which do not meet a 50% improvement may still be meaningful to patients.

E1332

ERYTHROPOIESIS STIMULATING AGENTS CAN IMPROVE ANEMIA IN PATIENTS WITH MYELOFIBROSIS TREATED WITH RUXOLITINIB

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Background: Anemia is common in patients with myelofibrosis (MF) and it is one of the main cause of symptoms in this setting. Erythropoiesis stimulating agents (ESA) have been used in MF but mostly small series and no randomized trials have been published so far. Anemia response rate ranged between 23 and 60% in different reports (Cervantes et al. BJH 2004; Cervantes et al., BJH 2006; Tsiara et al., Acta Haematologica 2007) and a larger study recently published by Cervantes group on 163 patients (Hernandez-SoludaJC. et al. JH 2016) showed a response rate of 50%. Ruxolitinib is currently approved for the treatment of intermediate 2 or high DIPSS/IPS risk MF and it is highly effective in reducing spleen size and controlling the symptoms of MF, thus resulting in a marked improvement in the patients’ quality of life (Verstovsek et al. NEJM 2012; Harrison C. et al. NEJM 2012) and possibly a prolonged survival (Cervantes F. et al Blood 2016). However, one of ruxolitinib main side effects is anemia, which occurs in 40% of the patients and can be a limiting factor for treatment tolerability and thus compliance and optimal dosage, mostly in the first weeks of treatment.

Aims: To evaluate the efficacy and safety of combination therapy with ruxolitinib and ESA.

Methods: We retrospectively evaluated 32 patients who received concomitant therapy with ruxolitinib and ESA. ESA (epoetin alpha or zeta or darbepoetin) were given off-label after obtaining patient written consent and local pharmacy approval. Erythroid response was defined as transfusion independence with normal haemoglobin (HB), transfusion decrease of >50% or sustained HB increase of >2g/dl, partial response as a sustained HB increase of 1-2g/dl.

Results: We included 32 patients diagnosed with MF, 23, 1% primary, 34, 6% secondary to PV and 42, 3% to TE. 20 patients (62, 5%) were male and median age at ESA start was 70 years (range 41-80). 87% of patients were at intermediate 2 and 13% at high risk according to DIPSS. Fifty-nine% of patients received epoetin alpha, 28% darbepoetin and 13% epoetin zeta. Median dose for epoetin alpha/zeta was 40000 U/week and for darbepoetin 150 mcg/week. Seven patients had started ESA treatment before ruxolitinib therapy, whereas 25 patients were ESA treatment naive after ruxolitinib start due to persistent or worsening of anemia. In particular, 5 were already RBC transfusion dependent before commencing ruxolitinib while 13 patients required red blood cell (RBC) transfusions only after treatment start. Overall ruxolitinib treatment worsened anemia leading to RBC transfusion requirement in 52% of patients. Median ESA start was 8 (range 4-10) months and median ESA dosage was 2.5 (range 1.2-10) mcg/kg/day. Thirty four patients (105 transfusion dependent. Median basal endogenous erythropoietin level was 58 U/l (range 8-146 U/l). Overall response rate was 87, 6%, with 68, 8% of erythroid response and 18, 8% of partial response. Median time to response and median

haematologica | 2017; 102(s2) | 547
E1333 COMPARING THE SAFETY AND EFFICACY OF RUXOLITINIB (RUX) IN PATIENTS (PTS) WITH DPSS LOW/INTERMEDIATE-1, INTERMEDIATE-2, AND HIGH-RISK MYELOFIBROSIS (MF) IN JUMP, A PHASE 3B, EXPANDED-ACCESS STUDY


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Background: RUX is a potent JAK1/JAK2 inhibitor that led to improvements in spleenomegaly and symptoms and increased overall survival in pts with intermediate (Int)-2 and high-risk MF by the Intromental Prognostic Scoring System (IPSS) in the phase 3 COMFORT studies. JUMP is a large, phase 3b, expanded-access trial in countries with no access to RUX outside a clinical trial and includes pts with IPSS Int-1–, Int-2–, and high-risk MF. To further evaluate RUX, we conducted an analysis assessing safety and efficacy of RUX by Dynamic IPSS (DIPSS) prognostic risk.

Aims: To compare the safety and efficacy of RUX in pts with DIPSS low/Int-1– vs Int-2– vs high-risk MF

Methods: Eligible pts had IPSS high- or Int-2–risk MF, or Int-1–risk MF and a palpable spleen (>25 cm). Starting dose was based on baseline platelet (PLT) count (5mg bid [≥50 to <100×109/L], 15mg bid [100-200×109/L], or 20mg bid [≥200×109/L]) and could be titrated during treatment. The primary endpoint was safety and tolerability of RUX. Changes in palpable spleen length and symptom scores were also assessed. DIPSS scores were determined using pt characteristics at baseline.

Results: Based on available pt data, DIPSS status was determined for 1840 of 2233 enrolled pts. JUMP included 893 low/Int-1–, 754 Int-2–, and 193 high-risk pts (primary MF, 57%, 63%, 62%) who started treatment ≥1 y before data cutoff (01 Jan 2016). Pts with higher-risk MF were older (62, 68, and 72 y), had lower Hb (<10 g/dL, 3%, 64%, 100%), and had higher blast counts (2%, 18%, 44%, 84%). Disease duration (50, 51, and 55 mo) and spleen size (12, 13, and 14.5 cm) were similar in all 3 groups. Most pts started at 20mg bid (68%, 57%, 59%) or 15mg bid (26%, 32%, 33%). Median exposure was 16, 11, and 9 mo; mean average daily dose was 30, 28, and 29mg. At data cutoff, most pts remained on treatment or had completed per protocol (70%, 57%, 51%). Main reasons for treatment discontinuation included adverse events (AEs; 15%, 17%, 15%), disease progression (6%, 11%, 11%), and death (2%, 5%, 11%). The most common hematologic grade 3/4 AEs were anemia (22%, 44%, 55%) and thrombocytopenia (11%, 18%, 25%), but these rarely led to discontinuation. Overall rates of nonhematologic grade 3/4 AEs were <2%, except for pneumonia (4.5%), pyrexia (2.3%), asthenia (2.2%), and dyspnea (2.2%). Infections in ≤5% of pts were pneumonitis (7.3%), urinary infection (6.1%), and nasopharyngitis (5.3%). Herpes zoster was reported in 4.8% of pts. At wk 48, 64% (226/355), 52% (121/232), and 50% (26/52) of pts had a ≥50% reduction from baseline in spleen length; 19% (68/355), 19% (43/232), and 23% (12/52) had 25%-50% reductions. Best response in spleen length by wk 72 is shown in the Figure; 69%, 57%, and 51% of pts achieved ≥50% reductions. Median time to response was 4.7 wk (2-75 wk), 5.3 wk (2.6-80 wk), and 8.1 wk (3.1-72.3 wk). From wk 4 to 48, 39%, 43%, 41%, and 48%, and 48% of pts achieved a clinically meaningful response on the FACT-Lym TS; proportions of responders on the FACT-Fatigue were 42%-49%, 46%-49%, and 55%-61%.

Figure 1.

Summary/Conclusions: RUX was safe and generally well tolerated. Interestingly, lower-risk pts received higher starting doses yet had lower rates of hematologic AEs. Additionally, lower-risk pts remained on treatment longer than higher-risk pts, with fewer discontinuations due to AEs. Lower-risk pts also achieved slightly better spleen size reductions and symptom improvement than higher-risk pts, suggesting that earlier RUX treatment may lead to greater benefits in pts with MF.

E1334 SAFETY AND EFFICACY OF RUXOLITINIB (RUX) IN PATIENTS WITH MYELOFIBROSIS (MF) WHO STARTED TREATMENT AT 10mg BID AND HAD THE DOSE UPTITRATED IN THE PHASE 3B EXPANDED-ACCESS JUMP STUDY


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Aims: Of a subset of JUMP pts provides information on this approach. ad hoc from clinical practice suggests that starting RUX at 10mg bid and subsequently uptitrating may reduce the risk of cytopenia development. An ad hoc analysis of a subset of JUMP pts provides information on this approach.

Aims: To assess the safety and efficacy of RUX at a starting dose of 10mg bid in pts with MF.

Methods: Pts with high-, Int-2-, or Int-1-risk MF were eligible. Int-1-risk pts had a palpable (≥5 cm) spleen. Protocol starting doses (5, 15, or 20mg bid) were based on baseline platelet (PLT) counts (≥50 to <100 x10^9/L, 100 to 200 x10^9/L, >200 x10^9/L, respectively). Although not per protocol, some pts started RUX at 10mg bid. The primary endpoint was safety. Secondary endpoints included changes in spleen length and symptoms.

Results: A total of 48 pts (primary MF, 60%) started RUX at 10mg bid ≥1 y before data cutoff (01 Jan 2016). Mean baseline characteristics were: median age, 65.5 y (range, 20-83 y); male, 44%; spleen length, 12.3 cm; time since diagnosis, 56.6 mo; hemoglobin (Hb), 11.2 g/L (<100 g/L, 33.3%); PLT count, 351 x10^9/L (<100 x10^9/L, 10.4%). Pt characteristics were similar to those of the overall population and did not indicate an increased risk of developing cytopenias. At data cutoff, most pts remained on treatment or had completed treatment per protocol (58.3%). Primary reasons for treatment discontinuation included adverse events (AEs), disease progression, and death (8.3% each). Overall, 41.7% of pts had dose modifications (AEs, 33.3%); 20.8% had interruptions (all due to AEs). Median exposure was 14.4 mo. The mean average daily dose was 25.8mg/day (SD, 10.1) and was comparable to those of pts starting at higher doses, leading to safety and efficacy outcomes consistent with those in the overall JUMP population. This alternative approach will be prospectively evaluated in anemic MF pts in the REALISE study (NCT02966635).

Background: JUMP is a phase 3b, expanded-access trial that assessed the efficacy and safety of RUX in pts with no access to RUX outside of a clinical trial. Pts received RUX at 5, 15, or 20mg bid per protocol. Increasing evidence from clinical practice suggests that starting RUX at 10mg bid and subsequently uptitrating may reduce the risk of cytopenia development. An ad hoc analysis of a subset of JUMP pts provides information on this approach.

Summary/Conclusions: A small cohort of pts in JUMP started at 10mg bid, and had the dose uptitrated during the first 8 wks to a mean average daily dose comparable to those of pts starting at higher doses, leading to safety and efficacy outcomes consistent with those in the overall JUMP population. This alternative approach will be prospectively evaluated in anemic MF pts in the REALISE study (NCT02966635).

E1335 HYDROXYUREA IS ASSOCIATED WITH SKIN TOXICITY IN MYELOPROLIFERATIVE NEOPLASMS: RESULTS FROM A PROSPECTIVE NON-INTERVENTIONAL STUDY

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Background: Until today, hydroxyurea (HU) remains the most commonly used cytoreductive drug in patients (pts) with classic myeloproliferative neoplasms (MPN), i.e. essential thrombocythemia (ET), polycythemia vera (PV), and myelofibrosis (MF). However, mucosal lesions, cutaneous ulcers, and pre-carcinomatous skin alterations such as actinic keratoses are being considered as potential side effects of HU.

Aims: We sought to investigate the occurrence of skin toxicity in MPN pts under HU compared to other (non-HU) cytoreductive drugs in routine clinical practice.

Methods: Classic MPN pts regularly presenting at the outpatient centers of the University Hospital of Ulm and Johannes Wesling Clinic Minden were included in our non-interventional study after having given informed consent. Skin alterations were evaluated prospectively between December 2010 and November 2016.

Results: In total, 151 MPN pts under cytoreductive therapy were included (ET, n=55; PV, n=55; MF, n=41). Primary MPN diagnosis was made between 1979 and 2012 at a median age of 55 years (range, 22-82). Median duration of the disease at baseline of the study was 6.3 years (0-32.6). Median prospective observation time for the total cohort within the study period was 5.3 years (0.4-6.2). Most frequently used cytoreductive drugs were HU in 120 pts, followed by ruxolitinib in 59, anagrelide in 39, and pegylated interferon-alfa (IFN-a) in 28 pts. Median cumulative HU exposure was 46 months (1-252), while the median cumulative treatment time for the corresponding cytoreductive drug was 24 months (1-267) [ruxolitinib: 22 months (2-64); anagrelide: 19 months (1-216); IFN-a: 64 months (1-267)]. Of 120 pts exposed to HU, 52 pts (43%) presented with skin abnormalities during the observational period occurring after a total HU treatment time of median 46 months (1-252). Sixteen of 120 pts (13%) discontinued HU due to skin toxicity such as skin ulcers (n=6), phototoxicity / erythrodermia (n=5), actinic keratoses (n=3), dry skin / xerostomia (n=2). Of note, four malignant skin diseases were reported under HU therapy (basal cell carcinoma, n=3; malignant melanoma, n=1). Although pts of the HU cohort were exposed longer to the drug compared to pts of the non-HU group, numbers of skin events in non-HU treated pts were as follows: n=5 under anagrelide (skin ulcers, n=2; allergic reaction, n=2; basal cell carcinoma, n=1), n=4 under IFN-a (local reaction after subcutaneous injection, n=3; actinic keratoses, n=1), and none under ruxolitinib. In 3/262 (2%) non-HU treated pts, occurrence of skin toxicity led to discontinuation of the corresponding cytoreductive drug. Interestingly, both skin ulcers as well as the single events ‘basal cell carcinoma’ and ‘actinic keratoses’ occurred under combination therapy with HU. Taken together, skin alterations occurred more frequently under HU compared to non-HU treatment (52/120 [43%] vs 9/126 [7%]; p=0.0001), and the same was true for treatment discontinuations due to skin toxicity (16/120 [13%] vs 3/126 [2%]; p=0.014).

Summary/Conclusions: According to our prospective observation, skin toxicity was clearly associated with HU treatment compared to other cytoreductive drugs. This resulted in a higher rate of HU treatment termination due to skin toxicity. However, median exposure time to HU was longer compared to non-HU treatment (52/120 [43%] vs 9/126 [7%]; p=0.0001), and the same was true for treatment discontinuations due to skin toxicity (16/120 [13%] vs 3/126 [2%]; p=0.014).

E1336 THE NEGATIVE PROGNOSTIC IMPACT OF BASOPHILIA, EOSINOPHILIA AND MONOCYTOSIS AT DIAGNOSIS IN PRIMARY MYELOPROLIFERATION

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Background: Primary myelofibrosis (PMF) is a myeloproliferative neoplasm (MPN) with a variable clinical presentation, from asymptomatic disease to rapidly progressive bone marrow failure and/or leukemic transformation; prognostic stratification using the DIPSS-plus score isolates patient cohorts with median survival ranging from 16 months to 185 months. The development of monocytosis during the course of PMF has been associated with a worse outcome, and absolute monocyte counts have been shown to be of prognostic value in other MPNs. Basophilia and eosinophilia are frequent findings in BCR-ABL-
positive MPNs, where they associate with an accelerated phase of disease, and seem to correlate with worse survival in myeloproliferative syndromes. However, the impact of these three findings at diagnosis in PMF remains unclear.

Aims: The aim of this work is to evaluate, at diagnosis, the prognostic impact of basophilia, eosinophilia and monocytosis in patients with PMF.

Methods: We identified all PMF patients diagnosed and followed-up in our Center between January 1st 2005 and August 31st 2016 who still fulfill PMF criteria under the WHO 2016 diagnostic revision, have synchronous bone marrow (BM) and peripheral blood (PB) analyses dating from the time of diagnosis, and have complete charts with no missing data. After the exclusion of reactive causes, monocytosis was defined as an absolute count (AC) >1.0 G/L, eosinophilia as an AC >0.6 G/L and basophilia as an AC >0.2 G/L.

Results: We studied 55 evaluable patients (73% male) with a median age at diagnosis of 70.1±11.7 years old. At diagnosis, 20% of patients had monocytosis, with no significant differences according to gender or age. The median overall survival (OS) in PMF patients with monocytosis was 27.3 months, and twice as long (46.4 months) in patients without. In this population, a new calculated cut-off of 0.75 G/L was better able to stratify patients according to survival with a specificity of 74.1% (95% CI: 53.7-88.9%), 32.7% of patients had an AC above the cut-off, with a median OS of 27.9 months, compared to 64.4 months for patients under the cut-off. We identified 12.7% of patients with eosinophilia at diagnosis, with no differences according to gender or age. PMF patients with eosinophilia had a five-fold lower median OS compared with patients without (6.1 vs 32.5 months, respectively). We obtained a new cut-off of 0.25 G/L of eosinophils, which separated patients with a specificity of 77.8% (95% CI: 57.7-91.4%); 29.1% of patients had an eosinophil AC above the cut-off, with a median OS of 25.6 months, and 32.5 months in patients without. With a new cut-off of 0.25 G/L of basophil, with a specificity of 88.9% (95% CI: 70.8-97.6%), 20.0% of patients had a basophil AC above the cut-off and a median OS of 19.7 months, compared to 46.4 months for patients under the cut-off. Considering the whole cohort, 61.8% of patients had normal monocye, eosinophil and basophil ACs; the median OS in these patients was 56.1 months, compared to 28.5 months in patients with an increase in at least one AC. Applying the new cut-offs, this difference in OS increased to 27.9 vs 64.4 months. Progression-free survivals were not calculated, since only 2 patients had BM- or PB-documented progression during follow-up.

Summary/Conclusions: We observed that the presence of monocytosis at diagnosis in PMF was associated with a halving of the median OS, while eosinophilia decreased the median survival to one-fifth; basophilia also associated with a reduction in survival, of approximately 20%. The application of specific cut-offs calculated for the cohort improved the differentiation and stratification of patients, with moderate to high specificity, further clarifying the negative prognostic impact of these three variables, at diagnosis, in PMF. Our results show that even simple, inexpensive and readily available parameters can be used to predict survival in PMF patients, and suggest that their integration into established scores could further increase the prognostic accuracy of the latter.

E1337

BLAST PHASE IN PH-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS: A SINGLE INSTITUTION RETROSPECTIVE ANALYSIS OF 85 PATIENTS

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Background: Classic Ph-negative myeloproliferative neoplasms (MPN) include essential thrombocythaemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF). Chronic evolution can lead MPN patients in chronic phase (CP) to develop acute myeloid leukaemia (AML), called blast phase (BP); this event occurs at rates of approximately 1% in ET, 4% in PV and 20% in PMF over the first decade from MPN diagnosis.

Aims: To evaluate differences in clinical features and outcome in 85 patients with BP in blast phase, according to MPN diagnosis and mutational profile.

Methods: We identified in our database all patients affected with ET, PV and PMF who developed acute myeloid leukaemia according to 2016 WHO criteria (≥20% blasts in bone marrow or peripheral blood) and for whom at least one DNA sample to define the mutational status of the three MPN driver genes (JAK2, CALR, MPL) was available. Telomere length (TL) was measured in lymphocytes using quantitative FISH. Telomerase activity was measured using the TRAP assay. Telomere lengths were compared using Student’s t-test. Telomerase activity was compared using the Mann-Whitney test.

Results: We retrospectively identified among 2902 consecutive patients affected with Ph-negative MPN 85 patients who progressed to BP, with a known molecular profile. JAK2 V617F mutation was present in 78/85 patients (91%), 8 CALR mutation, 1 MPL mutation, 1 JAK2/MPL mutation and 1 was triple-negative, 36 PV patients all JAK2V617F mutated, and 23 PMF patients of whom 17 were JAK2 mutated, 2 CALR mutated, 2 MPL mutated and 2 triple-negative. Median age at BP was 71.3 years (range 46.3-86), being higher in PV (median 73 years, range 46.3-84,7) compared to ET (median 66.8 years, range 54.4-86, P=0.031) and PMF (median 67.9 years, range 48.1-84.9, P=0.016). The complete blood count at leukemic evolution was not influenced by the initial diagnosis. At the time of BP, 31 out of 44 patients (70%) for whom cytogenetic analysis was available showed an abnormal karyotype (22 patients with complex karyotype or high risk aberrations), JAK2 mutated MPN can evolve into JAK2 wild type AML (9 of 28 patient with blasts DNA available), while CALR mutation was identified also in AML blasts in all 6 patients for which DNA was available. Time to leukemic evolution was shorter in PMF (35.3 months, range 3.6-141.1) compared to ET (176.7 months, range 14.4-362.3, P<0.001) and PV (129.1 months, range 17-367.8, P=0.001). According to chronic phase driver mutation, time to leukemic evolution was shorter in JAK2 V617F mutated PMF compared to CALR mutated PMF (30.6 vs 138 months, P=0.024), but not statistically different in JAK2 mutated ET compared to CALR mutated ET (123.4 vs 203.2 months, P=0.121). Outcome was dismal, indeed the median OS of patients with BP was 27.3 months, compared to 64.4 months in patients without. Progression-free survivals were not calculated, since only 2 patients had BM- or PB-documented progression during follow-up.

Summary/Conclusions: We observed that the presence of monocytosis at diagnosis in PMF was associated with a halving of the median OS, while eosinophilia decreased the median survival to one-fifth; basophilia also associated with a reduction in survival, of approximately 20%. The application of specific cut-offs calculated for the cohort improved the differentiation and stratification of patients, with moderate to high specificity, further clarifying the negative prognostic impact of these three variables, at diagnosis, in PMF. Our results show that even simple, inexpensive and readily available parameters can be used to predict survival in PMF patients, and suggest that their integration into established scores could further increase the prognostic accuracy of the latter.

E1338

TELOMERE LENGTH IS REDUCED IN ESSENTIAL THROMBOCYTHEMIA PATIENTS COMPARED TO AGE AND GENDER MATCHED HEALTHY CONTROLS

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Background: Essential thrombocythaemia (ET) is a clonal stem cell disorder, commonly diagnosed in the 6th or 7th decade of life. ET is associated with risk of thromboembolic events, hemorrhage, constitutional symptoms, progression to myelofibrosis and acute myeloid leukaemia. In over 85% of patients a clonal driver can be identified with mutations in JAK2 (50-60%), Calectulin (CALR) (25-30%) or the thromboxane receptor (MPL) (3-5%); the remainder of patients are termed “triple negative” (TN). Telomeres are non-coding regions of DNA consisting of thousands of repeated sequences (TTAGGG) and are considered central to chromosomal integrity and genomic stability. In healthy adults, telomere length (TL) progressively shortens with age; therefore, TL is considered a marker of ageing and genome stability. Hematopoietic cells in several hematological malignancies have been shown to be characterized by shortened TL.

Aims: Determine if there is TL shortening in patients with ET when compared to age and gender matched controls and establish the effects of cytoreductive therapy on TL.

Methods: 100 patients were included in the study (27 with CALR, 35 JAK2V617F and two MPL515W mutations. 36 patients were TN). Most patients were female (70% 70/100); median age was 45 years (range 20 - 86 years).

Figure 1.

Summary/Conclusions: Clinical phenotype and outcome of BP is not influenced neither by the diagnosis in chronic phase nor by the driver mutation; moreover the outcome is poor irrespective of treatment. PMF patients have a shorter time to BP than ET and PV patients; in PMF JAK2 V617F mutation is associated with a shorter time to BP compared to CALR mutation. The only potentially curative treatment is represented by allogeneic stem cell transplantation, but only a few patients can actually undergo this procedure.
TL was determined in peripheral blood mononuclear cells using a monochrome multiplex quantitative PCR based on the original methods described by Cawthon. All results were corrected for age and gender.

Results: Regardless of driver mutation status ET patients had significantly shortened TL compared with age and gender matched controls, p<0.0001. Considering individual mutation status these differences remained significant e.g. Cota R et al. 2009; Jak2V617F vs Placebo, p=0.007 and p=0.012 in TN patients. TL appeared more markedly short in the CALR cohort; for the 18 patients, whose TL was below the first centile, 55% (10/18) were CALR positive vs 28% (5/18) Jak2V617F positive vs 17% (3/18) who were TN. Concerning the potential impact of therapies 31/100 patients were treated with hydroxyacarbon (HC) (IFN) and 10/100 patients had prior exposure to HC. 34/100 were not on cytoreductive therapy. Remaining treatments were ruxolitinib (5), busulphan (4), anagrelide (1) and vorinostat (1). Independent of mutation status there was significant TL shortening in untreated patients, p=0.05; however, upon evaluating the impact of cytoreductive therapy on TL we noted that patients with HC treatment or with either current or prior exposure to HC had significantly shortened TL, p=0.0015 and p=0.0001 respectively. Strikingly, there was no significant difference in TL in IFN patients who had no previous exposure to HC, p=0.2 but those ET patients currently on IFN but with prior HC exposure still had shortened TL.

Summary/Conclusions: We document for the first time that ET patients, when compared to age and gender matched healthy controls, have shortened TL. This shortening is more pronounced in CALR and Jak2V617F positive patients. Concerning therapy whilst present in untreated patients TL shortening was more pronounced in HC treated patients indicating that there may be a therapy effect as has been observed after HC treatment in sickle cell disease. Of note IFN treated patients had more normal TL suggesting that the disease related TL effects may be reversed by this agent.

E1339

NUTRITION IN MYELOFIBROSIS: CORRELATES FROM THE COMFORT-1 STUDY

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Background: Nutritional status declines in most patients with myelofibrosis (MF). Sixty-seven percent of patients with MF lose weight over time and 27% of patients have a BMI decrease of at least one body mass index (BMI) category (Mesa et al. Blood. 2008;112(11):5224). MF also leads to deficient LDL and cholesterol levels compared to age matched controls (Mesa R A et al. Blood. 2007;110(11):2548). Both hypercholesterolemia (p<0.001) and weight loss>10% (p<0.0001) have been associated with decreased survival in PMF patients (Mesa et al. Blood 2009 114:3918). Jak2 inhibitor therapy has been found to improve nutritional markers including weight, cholesterol, albumin, and leptin compared to placebo in the COMFORT-1 study (Mesa et al. Clin Lymphoma Myeloma Leuk. 2015 Apr; 15(4): 214–221; Verstovsek et al. N Engl J Med 2012; 366:799-807). However, the correlation of these factors with other disease related variables and overall survival has not been established.

Aims: To evaluate the correlation, if any, between nutritional markers other variables collected in the COMFORT-1 study.

Methods: Data from the COMFORT-1 trial of ruxolitinib versus placebo was obtained from the Incyte for independent analysis. Data was analyzed for correlation with symptom burden and survival along with other variables. Symptom burden was assessed by the MF-SAF v2.0 (Mesa et al. Leuk Res 2009) for individual items and total symptom score (TSS).

Results: A total of 309 patients were available for analysis including 155 ruxolitinib and 154 placebo-treated patients. Baseline characteristics showed that the ruxolitinib BMJ was 24.9 (SD=4.5). Baseline demographic and other disease-related variables can be found in previous publications (Verstovsek et al. N Engl J Med 2012; 366:799-807). Correlatives: Baseline: For all patients at baseline, numerous correlations between baseline nutritional markers and markers of nutrition (Figure 1A) were identified. Total Symptom Scores (TSS) inversely correlated with albumin, cholesterol, alpha-feto protein, HDL, and serum erythropoietin levels. Baseline lepitin levels correlated with many items including BMI, albumin, cholesterol, LDL, erythropoietin, insulin and CRP. Placebo: For patients treated with placebo, changes in BMI inversely correlated with changes in CRP (r=-0.22, p=0.02). Correlatives were observed between BMI and TSS score, with cholesterol (r=0.87, p<0.001) and HDL (0.41, p<0.001). In addition to LDL, HDL change inversely correlated with TSS score (-0.24, p=0.02), and positively correlated with changes in bone pain (0.23, p=0.02), abdominal fullness (r=0.22, p=0.02), erythropoietin levels (0.27, p=0.01) and cholesterol levels (r=0.39, p=0.04). Ruxolitinib: Most correlations with nutritional and metabolic markers mirrored with baseline scores (Figure 1b). For ruxolitinib-treated patients, changes in Jak2V617F percentage status inversely correlated with changes in serum cholesterol (-0.26, p=0.008), lepitin (-0.38, p<0.0001), and LDL (-0.23, p=0.02). CRP changes were inversely correlated with change in cholesterol levels (-0.18, p=0.03).

Summary/Conclusions: We document for the first time that ET patients, when compared to age and gender matched healthy controls, have shortened TL. This shortening is more pronounced in CALR and Jak2V617F positive patients. Concerning therapy whilst present in untreated patients TL shortening was more pronounced in HC treated patients indicating that there may be a therapy effect as has been observed after HC treatment in sickle cell disease. Of note IFN treated patients had more normal TL suggesting that the disease related TL effects may be reversed by this agent.

E1340

IS THE SURVIVAL OF PATIENTS WITH ESSENTIAL THROMBOCYTEMIA BETTER IN THE LAST DECADE? RETROSPECTIVE ANALYSIS OF DATABASE OF LATIAL GROUP FOR THE STUDY OF NMP, PH NEGATIVE ET AND ETv MFC

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Background: To evaluate the prognosis of patients with Essential Thrombocytemia (ET) in the first decade of the century we assessed retrospectively the thrombosis free survival (TFS) and the overall survival (OS) of the patients diagnosed from 01/01/2000 to 31/12/2009 and collected on the database of our group.

Aims: Diagnosis of ET was performed with PVSG, WHO 2001 or 2008 criteria, according to the date of the first observation. The whole population of 757 patients was then divided in two groups: the first (group I) with the diagnosis performed between 01/01/2000 to 31/12/2005 (334 patients), presented a median follow-up of 111,9 months, the second (group II) diagnosed between

haematologica | 2017; 102(s2) | 551

Madrid, Spain, June 22 – 25, 2017
treatments can present skin side effects.

Aims: We have performed a dermatological review of a cohort of patients we follow-up at our center with the aim of assessing the cutaneous manifestations.

Methods: A randomized selection of patients with a diagnosis of essential thrombocythemia and polycythemia vera was performed. We create a specific consultation in which a detailed history of each patient (sex, age, diagnosis, signs and symptoms, treatments and its duration) as well as a deep dermatological examination was done. All data was collected in an Excel database and analyzed using the SPSS system.

Results: 63 patients (54 ET and 9 VP) were reviewed. The most frequent skin lesions were xenosis and/or keratosis pilars (76.2% patients), nail changes (41.3%), actinic keratosis (39.7%), hyperpigmentation of the skin (23.8%), pruritus (23.8%) and non-melanoma skin cancer (22.2%). In figure 1 we detail all the skin alterations that we have found.

Summary/Conclusions: Cutaneous involvement in MPNs is more frequent than expected and it is usually underdiagnosed. Some of these lesions could be prevented with the correct treatment of their pathology and adequate photoprotective measures. Some authors recommend an annual review by a dermatologist in a systematic way, especially in patients with higher risk factors: low phototype, high sun exposure, past dermatological history and prolonged cytoreductive therapy.

Figure 1.
presentation with thrombocytosis. No central pathology review was planned for this stage of the study.

Results: A total of 122 patients (58 males and 64 females; 54% >60 years of age; 65% with LDH ≥200 μM/mL) with a clinical history indicative of ET were initially assessed. A majority of patients (76%) presented with suspected ET within the last 5 years, likely because it was more difficult for clinicians to identify patients with SM by biopsy collected within a year of presentation with thrombocytosis if they presented more than 5 years ago. Out of 122 patients, 48 met the hemoglobin and/or leukocyte criteria outlined in the Carobbio algorithm, Figure. The BM examination was performed on 33 patients who met pre-specified criteria for the timing of bone marrow biopsy. About one third of the 33 patients had a diagnosis of ET and one third for PMF. While 2 of the remaining patients met criteria for PV, the rest were uncertain whether to represent true ET or early PMF, i.e., represented MPN-U (Figure 1).

Summary/Conclusions: Despite its methodological limitations, this initiative confirms that in real world clinical practice the Carobbio algorithm can be used to identify patients with early PMF and PMN-U classification among patients clinically suspected to have ET. It suggests a need for educational initiatives on using diagnostic algorithms to separate ET from PMF. It confirms the importance of hematologist-pathologist collaboration in reaching a final integrated diagnosis based on the WHO classification. These findings warrant investigation in larger prospective studies.

E1343
PK/PD MODELING COMPARING DIVIDED DOSING (200mg TWICE-DAILY (BID)) VS SINGLE DOSING (400mg ONCE-DAILY (QD)) OF PARACITINIB (PAC) IN PATIENTS WITH MYELOFIBROSIS (MF) ON THE PERSIST-2 PHASE 2 STUDY


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Background: MF is a life-threatening hematologic malignancy characterized by splenomegaly and debilitating constitutional symptoms. At the present, the JAK inhibitor ruxolitinib is the only therapy for patients (pts) with MF approved by the FDA. Proliferating cell nuclear antigen (PCNA) is a protein that has garnered regulatory approval. Although ruxolitinib has been shown to reduce splenomegaly and symptoms in pts with MF, it is associated with dose-limiting cytopenias, and not indicated for pts with platelets <50,000/µL. PAC is an oral JAK kinase inhibitor with specificity for JAK2, FLT3, IRAK1, and CSF1R. Using data from the phase 2 clinical trials, we have previously predicted that BID dosing would result in higher steady-state AUC and lower Cmax vs QD dosing, which may be associated with increased efficacy and comparable or improved safety. Thus, the phase 3 PERSIST-2 trial of PAC vs BAT (including ruxolitinib) in pts with MF and platelet counts ≤100,000/µL was planned to compare PAC 200mg BID to BAT 20mg QD.

Aims: Validate the clinical utility of PK/PD modeling to predict the PAC 200mg BID regimen in pts with MF treated in the PERSIST-2 trial.

Methods: Pts with MF and baseline platelet count ≤100,000/µL were randomized 1:1:1 to PAC 400mg QD, PAC 200mg BID, or BAT. Blood samples were collected from PAC-treated pts for PK and PD analysis at a prespecified subset of trial sites. Blood samples were collected on day 1 of week 1 (4 h post-dose), week 3 (pre-dose and 4 h post-dose), week 12 (pre-dose), and week 24 (pre-dose). At the remaining sites, blood samples were collected from PAC-treated pts for PK analysis only at weeks 12 and 24 (pre-dose).

Results: Blood samples were collected up to week 24 from 144 PAC-treated pts (78 BID, 64 QD). The PK of PAC was described by a 2-compartment model with first order absorption, first order elimination from the central compartment, and an absorption lag time. PAC QD was associated with higher Cmax and lower Cmin vs PAC BID (Table). Median PAC plasma concentrations during steady state weeks 12 & 24 were higher vs QD dosing by 10% and 15%, respectively. Median observed steady-state 4h concentration at week 3 (coincides with Cmaxss) was 12% higher with QD vs BID dosing. In an exposure-response analysis, with QD or BID dosing, no trends were detected for a relationship between observed Cminss and death, cardiac death, hemorrhagic death, hemoptysis, or gastrointestinal events (any grade, grade ≥2, or ≥3). Eleven (15%) and 13 (17%) PAC QD pts achieved SVR ≥50% and TSS reduction ≤50% at week 24, respectively, vs 16 (22%) and 24 (32%) PAC BID pts. Treatment with PAC BID but not QD showed a trend of increased SVR vs Cminss.

Table 1.

Summary/Conclusions: As predicted by PK modeling and simulations analyzing 200mg PAC 400mg BID was associated with higher Cmax and lower Cmin vs PAC 200mg BID in pts with MF from the PERSIST-2 trial. These differences appear to translate into an improved benefit/risk profile of PAC BID vs QD regimens.

E1344
ZMYM2-FLT3 IS A RARE, RECURRENT, CYTOGENICALLY CRYPTIC FUSION IN MYELOID/LYMPHOID NEOPLASMS WITH EOSINOPHILIA THAT IS RESPONSIVE TO FLT3 INHIBITION

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Background: Myeloid/lymphoid neoplasms with eosinophilia are characterised by diverse tyrosine kinase (TK) fusion genes, many of which can be effectively targeted by small molecule inhibitors. More than 70 TK fusion genes have been described, most of which are associated with visible cytogenetic abnormalities. However these fusion genes are rare, and the pathogenesis of the great majority of these fusions is unclear. As myeloid lymphoid neoplasms with eosinophilia (MPN-eo) remains unexplained. We hypothesized that some MPN-eo cases may be driven by hitherto undetected cryptic TK fusion genes.

Aims: To screen cases with MPN-eo for TK fusion genes and evaluate the significance of any novel fusions

Methods: PolyA RNA extraction from MPN-eo cases, RNA-Seq library preparation and 100bp paired-end sequencing was performed with multiplexing for a minimum of 75 million reads/sample using an Illumina HiSeq 2000. Bowtie, TopHat and TopHat-Fusion were used to align reads, resolve splice junctions, identify and filter potential TK fusions. Confirmation and screening of fusions were performed by RT-PCR and Sanger sequencing.

Results: Of 20 cases tested by RNASeq analysis, just one cryptic TK fusion was identified: ZMYM2-FLT3, predicted to arise as a consequence of an 8Mb inversion at 13q12. Unusually, both breakpoints fell within exons (ZMYM2 exon 20 and FLT3 exon 14, respectively) resulting in an in frame fusion. To confirm this might be recurrent, we analysed 165 additional cases by RT-PCR. One additional positive case was detected, with similar but not identical breakpoints to the initial case. Case 1, a 48 year old female, presented with leukocytosis (30x10⁹/L), eosinophilia (2x10⁹/L, elevated serum tryptase (37µg/l), splenomegaly and a hypercellular bone marrow (BM). Cyto genetics was normal and FIP1L1-PDGFRα, KIT D816V and JAK2 V617F were all negative and no pathogenetically relevant mutations were identified by a myeloid NGS panel (28 genes). After 10 months, she progressed to myeloid blast phase. Because the disease was resistant to AML-induction chemotherapy (FLAG-Ilda), an allo-HSCT was performed with a donor who was positive for ZMYM2-FLT3 fusion. Following this, complete remission was obtained with ZMYM2-FLT3 fusion negative graft vs host disease. As a consequence of chronic GvHD and septic shock, the patient died 6 months after allogeneic PBSC. The ZMYM2-FLT3 fusion gene was identified post mortem. Case 2, a 47 year old male, presented with eosinophilia (4.7x10⁹/L, 47% elevated serum tryptase (42µg/l) and a hypercellular BM. Cyto genetics was normal and FIP1L1-PDGFRα, KIT D816V and JAK2 V617F were all negative. There was no response on steroids or hydroxyurea. Following the finding of ZMYM2-FLT3 positivity, treatment with sunitinib was commenced. Blood counts started to improve from day 4 and normalized after 3 weeks. During a pause of 3 weeks due to pulmonary infection, leukocytosis/eosinophilia rapidly increased, but normalized again within weeks after restart of sunitinib. The patient has been maintained on sunitinib for 10 months (since re-start) and remains in complete hematologic remission.

Summary/Conclusions: ZMYM2 is the fourth gene reported to fuse to FLT3 in myeloid neoplasms but the first FLT3 fusion that is cytogenetically cryptic. As such, PAC 400mg BID may be amenable to treatment with FLT3 inhibitors and thus, although very rare, this fusion should be considered in the work up of MPN-eo cases. Due to their extensive diversity, we anticipate that RNAseq will become the method of choice to detect rare TK fusions.
BACKGROUND: Fibroblast Growth Factor Receptor (FGFR) inhibitors have demonstrated efficacy in solid tumors with FGFR pathway activation. INCB054828, a novel, highly selective FGFR1, FGFR2, and FGFR3 inhibitor, is being assessed for the treatment of several advanced malignancies (AACR 2015; Abstract 771). 8p11 myeloproliferative syndrome is an aggressive myeloproliferative neoplasm (MPN) associated with FGFR1 translocation on chromosome 8p11.

Aims: To describe the characteristics of a patient with FGFR1 activated MPN who achieved a complete hematologic and cytogenetic response with INCB054828 in an ongoing phase 1/2 trial (NCT02393248).

Methods: In this 3-part, phase 1/2 dose-escalation and expansion trial, eligible adults had any advanced solid tumor (parts 1 and 3) or malignancy with FGFRs/FR alteration (part 2). Patients had Eastern Cooperative Oncology Group performance status score ≤1 (part 1) or ≤2 (parts 2 and 3), and were refractory to prior therapy with no known effective standard therapy available to them. Patients received INCB054828 orally on a 21-day cycle (2-weeks on/1-week off) starting at 9mg QD and increasing to 15.9mg QD.

Results: This 51-year-old male patient with 8p11 translocated MPN diagnosis (currently the only patient with MPN enrolled in this trial), presented with abnormal white blood cell (WBC) count (eosinophils, 15%; peripheral blood [PB] blasts, 4%) and abnormal platelet count (68 x10^9/L). The patient had prior therapy with hydroxyurea. Bone marrow (BM) biopsy at study entry showed 95% myeloid blasts and 5% normal hematopoietic component (t(11.2;12) (PML/RARA), large metaphases, and European Myelofibrosis Network grade MF-1. After 6 weeks of treatment with INCB054828 at a dose of 9mg QD in part 2 of the study, WBC count normalized with disappearance of eosinophilia and PB blasts. BM biopsy demonstrated a normalization of bone marrow differential with 50% cellularity, 1% BM blasts, adequate tri-lineage hematopoiesis, MF-1 fibrosis, and a complete cytogenetic response. After 4 months of treatment the patient was hospitalized for pneumonia and study treatment was held. The patient progressed to AML shortly after therapy interruption, with BM blasts increasing to 83% and evidence of clonal evolution (47,XY; +8 (8,9) (11.2;33) (3)[48] idem, +19 (17]). The patient was taken off study at this time. (end of cycle 6) and subsequently achieved a complete remission on intensive chemotherapy with fludarabine, cytarabine, idarubicin, and allogeneic BM transplantation.

Summary/Conclusions: INCB054828 showed efficacy in this patient with FGFR1 activated MPN using a 21-day (2-weeks on/1-week off) regimen. Continuous treatment may be necessary to sustain response and avoid rebound as has been seen with other kinase inhibitor therapies. A phase 2 trial has been initiated to evaluate INCB054828 in patients with myeloid/lymphoid neoplasms with FGFR1 rearrangement (NCT03011372).

E1345
THE GRADE OF STROMAL CHANGES IMPACTS ON PROGNOSIS IN PATIENTS WITH PRIMARY MYELOFIBROSIS
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Background: Recently, a detailed grading system for the assessment of bone marrow stromal changes has been proposed in primary myelofibrosis, proved to be reproducible and adopted by the updated WHO 2016 classification.

Aims: In this study, we aim to evaluate any possible prognostic implications of this grading system in a series of patients with primary myelofibrosis.

Methods: The study involved 122 consecutive patients with primary myelofibrosis diagnosed between 1998 and 2015 at the Oncohematology Division of the Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico of Milan, for whom bone marrow trephine biopsy (more than 1 cm in length) performed at the time of first observation was available, together with complete clinical, laboratory and follow-up data.

Results: Reticulin myelofibrosis (MF), collagen deposition (Co) and osteosclerosis (Ost) were evaluated and graded from 0 to 3 in the bone marrow trephine biopsies for each patient at diagnosis. In detail, the stromal changes were graded as follows: bone marrow fibrosis (MF): 0 in 9 cases, MF-1 in 60, MF-2 in 31 and MF-3 in 22; collagen deposition: Co-0 in 64 cases, Co-1 in 23, Co-2 in 21 and Co-3 in 14; osteosclerosis: Ost-0 in 72 cases, Ost-1 in 24, Ost-2 in 19 and Ost-3 in 7. Patients’ population was composed of 56 males and 66 females (MF=[1,2]) with a median age at diagnosis of 68 years (range 30–85). Clinically, at presentation, anaemia and hemoglobin values less than 10 g/dL was present in 20 (16%) patients, leukocytosis more than 25 x10^9/L was identifiable in 4 (3%) patients, and platelets count less than 100 x10^9/L in 7 (6%) cases. JAK2V617F mutation was detected in 81 cases (66%). Among the remaining 41 JAK2-negative patients, 4 and 27 carried MPL and CALR mutations, respectively; 10 out of 122 resulted “triple-negative”. According to the International Prognostic Scoring System, 38 cases were stratified as low risk, 51 as intermediate-1 risk, 21 as intermediate-2 risk, and the remaining 12 as high risk.

By the time of the analysis, 21 (17%) patients had died: leukemic evolution occurred in 14 (11.5%) patients, whereas thrombotic or hemorrhagic events occurred in 25 (20.5%). Subsequently, a comprehensive grade of bone marrow stromal changes ranging from 0 to 9 allows us to distinguish 68 (72%) cases with low-grade stromal changes (total score: 0-4) and 34 (28%) with high-grade stromal changes (total score: 5-9).

Clinically, patients with high-grade stromal changes presented more frequently with anaemia, thrombocytopenia, leukocytosis, peripheral blood blasts and increased lactate dehydrogenase levels. The grade of bone marrow stromal changes resulted strictly associated with the International Prognostic Scoring System and the overall mortality (low-grade: 10 dead out of 88 vs high-grade: 11 dead patients out of 34; p=0.013). Finally, the grade of bone marrow stromal changes was effective in discriminating the overall survival of the patients with low-grade and high-grade stromal changes (Log-Rank test: p=0.0002).

Summary/Conclusions: A detailed evaluation of the bone marrow stromal changes has important prognostic implications and can be used at diagnosis in the clinical stratification of the patients affected by primary myelofibrosis. Further studies are needed to test if the prognostic significance of this grading system remains during the follow-up.

E1346
INCREASED RISK OF INFLAMMATORY BOWEL DISEASE IN PATIENTS WITH PHILADELPHIA NEGATIVE CHRONIC MYELOPROLIFERATIVE NEOPLASMS
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Background: Studies reveal that patients with inflammatory bowel disease (IBD) may have increased risk of haematological cancers. Moreover, Philadelphia negative chronic myeloproliferative neoplasms (MPNs) have previously been associated with autoimmune diseases, including IBD. Nevertheless, to our knowledge, the risk of IBD has not been investigated in patients with MPN.

Aims: We undertook a nationwide population-based matched cohort study, and investigated the risk of IBD in patients with MPN.

Methods: We used valid Danish national registries, covering more than 5 million individuals, and included all patients diagnosed with either essential thrombocytthemia (ET), polycythaemia vera (PV), myelofibrosis (MF), or unclassifiable myeloproliferative neoplasm (MPN-U) during the study period; including 37 ET patients, 28 PV patients, 1 MF patient and 14 MPN-U patients. During a total risk time of 45,241 years, the rate of IBD per 1000 person years at risk was 1.8 (95% confidence interval [95% CI]:1.4-2.2) for the MPN patients. The corresponding rate for the 81,326 comparison was 0.8 (95% CI: 0.7-0.9). The 10-year risks of IBD for MPN patients and comparisons were 0.8% (95% CI: 0.6-1.0) and 0.4% (95% CI: 0.4-0.5), respectively. The overall HR of IBD was 2.4 (95% CI: 2.1-2.9) for MPN patients, with HRs of 2.6 (95% CI: 2.1-3.2) for ulcerative colitis and 2.4 (95% CI: 1.7-3.4) for Crohn’s disease. The risk of IBD was increased 2 to 3 fold among ET, PV and MPN-U patients, with HRs of 2.8 (95% CI: 2.1-3.7) for ET patients, 2.1 (95% CI: 1.6-2.7) for PV patients and 2.2 (95% CI: 1.3-3.7) for MPN-U patients.

Summary/Conclusions: Patients with MPN are at increased risk of IBD compared to the general population. The absolute risks of IBD are low, but abdominal discomfort may in few patients be caused by underlying IBD.
ESSENTIAL THROMBOCYTHESIS WITH AQUAGENIC PRURITUS: AN ENTITY WITH MORE AGGRESSIVE CLINICAL AND BIOLOGICAL PROFILE AT THE DIAGNOSIS AND A HIGH MORBIDITY DURING THE FOLLOW-UP
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Background: Polycythemia vera (PV) and essential thrombocythemia (ET) are Ph-negative myeloproliferative neoplasms in which arterial or venous thromboses and phenotypical evolutions (leukemia, myelofibrosis) are the most recurrent complications. Aquagenic pruritus (AP), induced by water contact, is a typical symptom of PV. However, we showed recently that ET patients also suffered from AP with clinical characteristics quite different from those observed in PV patients. In 2008, the presence of AP was associated with a lower risk of arterial thrombosis in PV patients.

Aim: It seemed particularly interesting to analyse the clinical relevance and the prevalence of the presence of AP in ET patients for such a risk.

Methods: In this study, we used the OBENE observatory (Observatoire Brestois des Épistélas myeloprolifératives), a register of MPN patients followed in our University Hospital in which biological and clinical data of 396 ET patients have been collected. This register was approved by a local ethical committee and registered in clinicaltrials.gov (NCT02897297). To avoid masked polycythemia Vera diagnostics, all JAK2 positive cases were tested for isotopic red mass cells if appropriate.

Results: Among the 396 ET patients, 42 (10.6%) suffered from AP. Interestingly, the median age at diagnosis of these patients was lower (51.6 vs 63.8%, p<0.0001). Furthermore, they presented more symptoms as erythrocytosis, hyperviscosity, constitutional symptoms and splenomegaly (p<0.01). ET patients with AP were more proliferative (more polycythemic but less thrombocytophenic) and were more difficult to treat (2.2 vs 1.1 treatment lines, p=0.005). Concerning the occurrence of thrombotic events (arterial or venous) at diagnosis, no significant difference between patients with or without AP was found. In contrast, the presence of AP induced an increase of thrombotic events during the follow-up (30.9 vs 17.2%, p=0.03). But surprisingly, these events appeared in the delayed timing. The arterial/venous rate of thrombotic events was also different with 50/50 vs 25/15. Furthermore, we observed that about one-third of the patients with AP had phenotypic evolutions against 13.3% in the other group (p=0.0007); the most frequent evolutions were PV and secondary myelofibrosis (16.7 vs 5.4%, p=0.005 and 19 vs 4.8%, p=0.0003, respectively). Concerning the overall survival of the patients, we have noted that there was less death in the group with AP than without AP (11.9 vs 32.5%, p=0.006) in spite of a longer follow-up (12.1 vs 7.7 years, p=0.002).

Summary/Conclusions: AP is classically associated to PV. But we confirmed here that AP is also present in ET. Furthermore, ET patients suffering from AP were more proliferative, more symptomatic at diagnosis but had also higher risk of thrombo-phenotypic complications than ET without AP. Despite that these patients have a higher overall survival. So, the presence of AP in ET patients with ANAGRELINE RESPONSE ACCORDING TO THE MOLECULAR PROFILE: SOMETHING TO CONCLUDE ON THE MECHANISM OF ACTION OF THE DRUG IN MYELOPROLIFERATIVE NEOPLASMS (MPN)? M. Montero1, T. Knight1, M. Dominguez-Z1, E. Canillo1, F. Marquez-V, E. Escamilla1, P. Guerrero1, M. Suito, A. Blum1, N. Alkadi1, J. González2, J. Falantes2, N. Rodriguez1, M. Martin1, I. Espigado1, J. Pérez-Simón1
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Background: Anagrelide is a useful drug in the control of thrombocythemia in MPN. Although it is known that in therapeutic levels it primarily influences in the post-mitotic phase of megakaryocytic development interfering with its complete maturation, its mechanism of action is still ill-known.

Aims: The purpose of the diagnosis of MPN due to the discovery of driver mutations (JAK2, calreticulin and MPL) leads us in the present study to correlate them with the response to anagrelide in a group of patients treated with this drug, investigating the possible interference in the referred biological pathways.

Methods: 150 patients with MPN diagnosed in our centre between 1993 and 2015 were studied. The median age was 49 years, with 19 patients older than 60 years. 83% were female and 17% were male. The diagnosis was initially carried out based on the WHO criteria 2008 and subsequently reviewed the medical records with the new criteria of 2016. A molecular study on peripheral blood samples was carried out using quantitative allelic-specific PCR technique for JAK2, qualitative for MPL (L515V mutation) and Sanger sequencing of exon 9 for calreticulin. Type 1 mutation was considered at 52 bp deletion and type 2 at 5 bp insertion. In all patients, the goal of anagrelide therapy was to control thrombocytosis (platelet count below 600x10^9/L), with dosage within the range of efficacy and safety recommended in the datasheet. The results were analysed with the statistical software SPSS vs 15.0.

Results: 80.5% of the patients were diagnosed with ET, 12.5% of PV, 3.5% of myelofibrosis and 3.3% of unclassifiable MPN. 59% of the patients had a V617F JAK2 mutation, with allelic load higher than 20% in 47.5% of the cases. 28.5% presented mutation in calreticulin; of which 50% were type 1 and 50% type 2. Only one patient had a mutation in MPL (2%), the remaining 6% being classified as “triple negative”. The median daily dose of anagrelide received was 1.5mg. 17.5% of the patients required more than 2mg for an adequate control, half of them being positive for mutations in calreticulin and the other 50% of the mutation V617F. 2% of patients with allelic load higher than 20% and 26% of the patients received daily dose of 1mg, being 70% positive for the mutation V617F/JAK2 with allelic load lower than 20%, although there were no statistically significant differences between the groups according to the mutational profile. 16% of patients discontinued treatment due to toxicity, with the most common adverse effects being mild (headache and palpitations).

Summary/Conclusions: Patients requiring higher doses of anagrelide present mutations in calreticulin or JAK2 V617F allelic load higher than 20% and patients with lower allelic load having greater sensitivity to the drug, with no statistically significant differences. It is possible that the first situation is associated with a greater pre-mitotic deregulation in the megakaryocyte where the drug does not interfere whereas the second one could be related to anagrelide interference through the JAK2 pathway in post mitotic maturation although larger exploratory studies are required.

E1349
LONG-TERM AND LOW-DOSE BUSULFAN IS SAFE AND EFFECTIVE IN ELDERLY PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA (ET) RESISTANT OR INERT TO HYDROXYUREA (HU): A PROSPECTIVE, MULTICENTRIC, TECHNICAL, RANDOMIZED, CONTROLLED STUDY
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Background: Therapeutic options for elderly patients (pts) with Essential Thrombocythemia (ET) resistant or inert to hydroxyurea (HU) are limited. Busulfan (BU) is a possible second-line treatment, but conventional schedule
(starting dose of 14mg/week up to obtain the complete hematological response (CHR) is associated with high risk of leukemic transformation and second malignancies.

**Aims:** We analysed efficacy, toxicity, risk of myelofibrosis (MF) and leukemic evolution in 31 of 352 ET pts collected in our database, treated with an alternative long-term schedule of BU, defined by low-starting dose (4-6mg/week) up to CHR (evaluated according to ELN response criteria), followed by dose de-escalation overtime.

**Methods:** Non parametric tests, such as Mann-Whitney, Pearson Chi-square and Fischer's exact tests, were used for statistical analysis of continuous and categorical variables. Survival curves were calculated by Kaplan-Meier method and compared with Log-rank (Mantel-Cox) test.

**Results:** 27/31 pts were evaluable for analysis (8 male, 19 female). Median age at diagnosis and at BU start were 71.3 and 79 years (yrs) respectively. We found these driver mutations: JAK2V617F in 15 pts (55.6%), Calreticulin in 8 pts (29.8%) and MPL in 1 patient (3.7%); 3 pts (11.1%) were triple negative. IPSET score at diagnosis was low-intermediate in 17 (83%) and high in 10 (37%) pts. 26 pts started BU as 2nd line treatment: 11 (42.3%) were intolerant and 15 (57.7%) were resistant to HU respectively. Only one received BU as 1st line treatment. They received BU for a median time of 47.67 months (range: 1.48 – 94.42). The median cumulative BU dose was 453mg (range: 32-1032), 26/27 pts (96.3%) obtained CHR, after a median time of 191 days. 6 pts (22.2%) obtained CHR after a median time of 216 days. Drug-related side effects appeared in 3 pts (11.1%). During time of analysis 5 pts (18.5%) died.

**Summary/Conclusions:** Our experience with an alternative long-term and low-dose BU administration is safe and effective in elderly patients with ET.

**E1352**

**DIFFERENCES IN JAK2V617F POSITIVE PATIENTS WITH AND WITHOUT THROMBOSIS ACCORDING TO DIAGNOSIS, AGE, SEX AND V617F ALLELE BURDEN**

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**Background:** Thrombosis is one of the most frequent events in Ph(-) myeloproliferative neoplasms and the reasons for that are still under investigation.

**Aims:** The aim of this study was to find out if there is difference in frequency and type of thrombosis in JAK2 V617F positive patients according to their diagnosis, age, sex and V617F allele burden.

**Methods:** One hundred and eighty two JAK2 V617F positive patients diagnosed with polycythemia vera (PV) N=63, essential thrombocythemia (ET) N=83, and primary myelofibrosis (PMF) N=36 were included in the study.

Patients in each group were additionally divided according to sex, age at diagnosis and first thrombosis. V617F allele burden was quantified in peripheral blood granulocyte DNA by real time PCR established by Larsen et al. Br J Haematol 2007;136:745.

**Results:** Among 182 patients observed, 66 (36%) experienced thrombosis, with arterial thrombosis being twice more frequent than venous thrombosis in all 3 studied groups. In ET group there was statistically significant difference in sex distribution (proportion of females=0.71), p<0.001. Statistically significant difference in age at diagnosis was observed between ET and PV/PMF patients without thrombosis (p=0.001); the youngest patients were those in ET group.

The age at diagnosis of ET patients with thrombosis (65 years, range 23-92) was statistically different compared to ET patients without thrombosis (50 years, range 21-83), p=0.002. Our study showed that V617F allele burden in patients without thrombosis was statistically significantly different between ET (17.2%, range 4.2-55.2) compared to PV (43%, range 1.7-99.9) and PMF (37.1%, range 1.4-90.7), p<0.001. The same statistically significant difference for V617 allele burden was established in patients with thrombosis between ET patients (19%, range 1.4-84.5) and PV and PMF patients (42.5%, range 8.9-97.2 and 48.8%, range 1.6-99.8, respectively), p<0.001.

**Summary/Conclusions:** Our results confirm that arterial thrombosis is more frequent than venous thrombosis in JAK2 V617F positive patients. Female sex was prevalent only in ET group. The age at diagnosis in all studied groups was similar except for ET patients without thrombosis. There was no difference in the frequency and type of thrombosis among ET, PV and PMF patients with high heterogeneity in V617F allele burden between all studied groups regardless of the occurrence of thrombosis.
Non-Hodgkin & Hodgkin lymphoma - Biology

E1355
PROTECTION AGAINST DEVELOPMENT OF B CELL LYMPHOMA BY TETRASPANIN CD37
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Background: B cell non-Hodgkin lymphoma, worldwide the most common hematological malignancy, remains a clinical problem. The molecular events leading to B cell lymphoma are only partially defined. CD37 is a member of the tetraspanin superfamily that is highly expressed on mature B cells and is required for optimal GC function and long-lived antibody production.

Aims: We investigated the function of tetraspanin CD37 in the development of B cell lymphoma.

Methods: A combination of studies was performed in mouse models (CD37-/-, IL-6-deficient mice), and studies of DLBCL patient material using biochemical, immunological, genetic and microscopical approaches.

Results: We provide evidence that deficiency of CD37 induces the development of B cell lymphoma in vivo. Cd37-deficient mice develop germinal center-derived B cell lymphoma in lymph nodes and spleen with higher incidence than Bliz2-transgenic mice. We discovered that CD37 interacts with SOCS3, and when absent drives tumor development through constitutive activation of the IL-6 signaling pathway. The importance of the IL-6-pathway was confirmed by investigating Cd37xIl6 double knock-out strains that were fully protected against lymphoma development. Our unpublished data shows discovery of inactivating CD37 mutations in patients with DLBCL. Importantly, loss of CD37 on neoplastic cells in patients with diffuse large B cell lymphoma (DLBCL) is directly correlated with activation of the IL-6 signaling pathway and with worse progression-free and overall survival.

Figure 1.

Summary/Conclusions: Together, this study identifies tetraspanin CD37 as a novel tumor suppressor that directly protects against B cell lymphomagenesis, and provides a strong rationale for blocking the IL-6 pathway in patients with CD37-negative B cell malignancies as therapeutic intervention.

E1354
CONCOMITANT DUAL ABLATION OF BLIMP1 AND P53 IN B-CELLS AS A NOVEL IN VIVO MODEL FOR HIGH-GRADE B-CELL LYMPHOMA
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Background: B-Lymphocyte-Induced Maturation Protein-1 (BLIMP1)-1 and p53-inactivation contributes to the pathogenesis of a wide spectrum of malignancies, including diffuse large B-cell lymphomas. Nevertheless, there is lack of in vivo models that may be used for a better understanding of the biology and genomics of high-grade B-cell lymphomas characterized by dual loss of both BLIMP1- and p53.

Aims: 1) To develop and characterize a transgenic mouse model of BLIMP1- and p53 dual loss in B cells; 2) To provide an in vivo model that mirrors human ABC-DLCL phenotype.

Methods: Cre recombinase under the control of CD19 promoter (C57BL/6 CD19CreCre) mice were crossed with either C57BL/6 BLIMPflox/flox or C57BL/6 p53flox/flox mice to achieve deletion of BLIMP or p53, respectively, in B cells. Secondly, CD19CreCre BLIMPflox/flox mice were crossed with CD19CreCre p53flox/flox to achieve dual deletion of BLIMP and p53 in B cells (CD19CreCreBLIMPflox/flox p53flox/flox, referred as CD19Bl-/p53-). Transgenic experimental mice (CD19Bl-/p53-) where characterized for clonal B cell infiltration using immunohistochemistry, flow cytometry, Southern Blotting, whole exome sequencing. MTT assay was used to test BTK-inhibitor-dependent cytotoxicity using CD19/Bl-/p53-derived B220 cells.

Results: CD19/Bl-/p53- mice presented with diffuse lymphadenomegalies, splenomegaly, hepatomegaly (100%, 90.3% and 77.4%, respectively). Other clinical manifestations included presence of ascites and hind limb paralysis (12.5% and 19.3%, respectively). The CD19/Bl-/p53- showed increased abdominal mass compared to Bl-/p53- mice non-expressing the CD19/Cre recombinase, CD19/p53-, or CD19/Bl-/ (363, 469.5, 460.5, and 770 days, respectively). H.E. staining of CD19/Bl-/p53- derived lymph nodes, defined a nodal architecture with a monomorphic population of large sized atypical lymphoid cells, multiple bone marrow sinusulations. Indeed, only mice transplanted with naive CD37- and p53 was also observed. Features were compatible with a high-grade lymphomas. IHC analysis confirmed positivity for B220 staining (TdT, Bcl6, CD138 and CD4, CD8 negative). Tumors were confirmed to be B220+IgM+, with either Igk- or Igλ lambda-restriction as demonstrated by flow cytometry; and either mono- or bcl-1 staining demonstrated by Southern blotting. Variant derivation of the mouse tumor was performed from B220+ selected cells obtained from pathological lymph nodes of CD19/Bl-/p53- mice and identified 143 SNVs. Non-synonymous somatic mutations were mapped on genes involved in the regulation of focal adhesion, PDGF signaling, p53 downstream pathway, and lipoprotein metabolism. B220+ cells selected from CD19/Bl-/p53- derived lymph nodes were implanted s.c. into recipient SCID/Bg mice, and presented with 100% engraftment, with a monomorphic lymphoid infiltration of B220+ and Igλ+ cells. B220 positive cells were selected from the s. q. tumor and intravenous injected into recipient SCID/Bg (n: 10) and BL6 mice (n: 10). Engraftment was demonstrated in all the mice, where hepatomegaly and splenomegaly were also observed. Infiltration of B220+ cells was documented within bone marrow, liver and spleen. Finally, we found that B220+ cells selected from lymph nodes harvested from CD19/Bl-/p53- mice were sensitive to ibrutinib.


E1355
IDENTIFICATION AND CHARACTERIZATION OF THE LYMPHOMA INITIATING CELL (LIC) POPULATION IN AN ALCL MOUSE MODEL
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Background: In 60% of anaplastic large cell lymphoma (ALCL) patients a t(2;5) translocation is found, which results in NPM-ALK fusion gene expression and constitutive activation of the ALK tyrosine kinase. Immunophenotypic characterization of human ALCLs revealed highly CD30-positive cells of T- or null-cell-origin.

Aims: However, the origin of the lymphoma initiating cell population as well as NPM-ALK signal transduction in course of the disease remains unclear and needs to be characterized.

Methods: In this regard, we established a retroviral murine bone marrow transplantation model resembling human ALCL. Therefore we use an inducible Cre/loxP system where NPM-ALK expression is restricted to early T-cells. We infected bone marrow of Lck-Cre transgenic mice with our MSCV-Stop-NPM-ALK-ires-EGFP vector and transplanted it into lethally irradiated irradiated CD4−/CD8− mice. With a latency of 4-5 months, mice developed CD30-positive lymphomas and died from neoplastic T-cell infiltration of lymphatic organs and bone marrow.

Results: Immunophenotypic analysis confirmed T-cell origin of the lymphomas with a heterogeneous compound of all T-cell stages with mainly CD4+CD8+ double positive T-cells including all DN T-cell subpopulations as well as hematopoietic stem cells and lymphatic precursors. Staining of the T-cell subpopulations demonstrated high NPM-ALK expression in immature CD4+CD8+ double negative T-cells and undifferentiated CD4+CD8+ double positive T-cells with highest expression of proliferation marker Ki67 as well as the activation marker PDGF. In course of the disease NPM-ALK expression and constitutive activation of the ALK tyrosine kinase. Immunophenotypic characterization of human ALCLs revealed highly CD30-positive cells of T- or null-cell-origin.}

Summary:

identification and characterization of the lymphoma initiating cell (lic) population in an alcl mouse model

haematologica | 2017; 102(s2) | 557

Madrid, Spain, June 22 – 25, 2017
Aims: be associated with a distinctive LGL immunophenotype and/or indicative for and secretion) and cell-mediated mechanisms.

Summary/Conclusions: In summary, our results highlight the existence of a lymphoma initiating stem-cell-like population originated within the DN3/DN4 lymphoma cell population in a highly relevant NPM-ALK positive CD30-expressing ALC1 mouse model, thereby giving the opportunity to test the eradication of the LIC with established and new therapeutic approaches.

E1356
HSP110 SUSTAINS MYD88-DEPENDENT NFkB SIGNALING IN ACTIVATED B CELL DIFFUSE LARGE B CELL LYMPHOMA
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Background: Diffuse large B cell lymphoma (DLBCL) is an aggressive lymphoproliferative disorder of B lymphocytes accounting for 30% of adult Non Hodgkin Lymphoma (NHL). Among DLBCL, Activated B Cell – DLBCL (ABC-DLBCL) is the most aggressive form and has a poor prognosis. Heat-shock proteins (HSPs) are molecular chaperons highly expressed in cancer cells and implicated in resistance to radio- and chemotherapy. Therefore, HSPs are envisioned as therapeutical targets in many cancers. Among the different HSPs, HSP110 has been recently identified as a pro-survival factor in germin center-derived DLBCL (GC-DLBCL), through stabilization of the GC-DLBCL oncogene Bcl-6.

Aims: Here, we have explored if HSP110 could also be involved in the survival of the most aggressive form of DLBCL

Methods: The study was performed with ABC-DLBCL patient samples and several cell lines. SHRNA specific for HSP110 was introduced through a lentiviral vector designed to infect highly efficiently non-permissive B cell lines. Through FAS LIGAND SECRETION

STAT3 ACTIVATION MEDIATES CD8+/CD16+/CD56- T-LGLL NEUTROPENIA

E1357
STAT3 ACTIVATION MEDIATES CD8+/CD16+/CD56- T-LGLL NEUTROPENIA THROUGH FAS LIGAND SECRETION
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Background: T-cell large granular lymphocyte leukemia (T-LGLL) is a rare chronic lymphoproliferative disorder characterized by the clonal expansion of CD3+ Large Granular Lymphocytes (LGL). In addition to the most common CD16+/CD56- T-LGLL, other subtypes are described in up to 12% of patients, including CD4+ T-LGL leukemia, less frequent LGL proliferations with CD4+/CD8-/+dim phenotype (CD4+ T-LGLL leukemia) exist, which are characterized by indolent clinical course. Somatic STAT3 mutations determining constitutive activation have been recently reported in a proportion of approximately 40% of patients, with no clear correlation with the occurrence of neutropenia, whose pathogenesis is currently multifactorial, comprising both tumoral (i.e. soluble Fas ligand and secretion) and cell-mediated mechanisms.

Aims: The aim of this work was to evaluate whether 1) STAT3 mutations might be associated with a distinctive LGL immunophenotype and/or indicative for symptomatic disease and 2) STAT3 activation is directly related to the development of neutropenia.

Methods: A cohort of 101 patients affected by T-LGLL according to WHO criteria were screened for STAT3 mutation by Sanger sequencing and PCR ARMS assay. All the samples were analysed by flow for CD3, CD4, CD8, CD16, CD56 and CD57 antigen. STAT3 tyr 705 levels were studied by Western blot. FAS ligand mRNA levels were analysed by RT-PCR Assay.

Results: By flow we observed that 65 out of 101 patients (67.3%) were characterized by CD3+/CD8+/CD4- expression (CD8+ T-LGLL), while the remaining 33 patients (32.7%) were CD3+/CD4+/CD8+-/dim/neg (CD4+ T-LGLL). All STAT3 mutated (n=38) and almost all neutropenic (38 out of 39) patients belonged to CD8+ T-LGLL leukemia (n=68), while among CD4+ T-LGL leukemia (n=33) no STAT3 mutated and only one neutropenic patient (1 out of 33, 3%) was found. Among CD8+ T-LGLL, immunophenotypic signature CD16+/CD56- was both associated to the presence of neutropenia and STAT3 mutation (37 out of 41, 90.2%, c2=49.5, p<0.0001 and 37 out of 41, 90.2%, c2=49.5, p=0.001 respectively). Furthermore, by western blot we showed that high STAT3 lysine phosphorylation observed in LGL obtained by CD8+ T-LGLL patients belonging to CD16+/CD56- subgroup was significantly higher as compared with other immunophenotypic groups. Provided this relationship between STAT3 mutation/activation and neutropenia, by RT-PCR we analysed Fas ligand expression, showing higher transcription levels in CD16+/CD56- CD8+ T-LGLL patients as compared to the not neutropenic patients belonging to the other immunophenotypes, both CD8+ T-LGLL and CD4+ T-LGLL (7.66±0.87, 2.45±0.22 and 2.35±0.28 arbitrary units, respectively; p<0.01). To confirm this relationship, in patient's PBMCs treatment with STAT3 inhibitor Static decreased both STAT3 phosphorylation and Fas ligand transcription as compared to the untreated conditions. In addition, IL-6 and IL-15 stimulation (which are known STAT3 activator) increased Fas ligand transcription levels (1.59- and 2.01-fold after IL-6 and IL-15, respectively) which is prevented by concomitant Static treatment.

Summary/Conclusions: Our results provide evidence that STAT3 mutation and activation is mostly restricted to neutropenic CD8+ T-LGLL patients equipped with the CD16+/CD56- signature. The relationship between STAT3 activation and neutropenia FAS ligand related further supports to approach STAT3 inhibition as therapeutic strategy in symptomatic CD8+ TDLBCL+CD56- T-LGLL patients, obtaining the dual results of inducing apoptosis in leukemic lymphoma cell population in a highly relevant NPM-ALK positive CD30-expressing ALCL mouse model, thereby giving the opportunity to test the eradication of the LIC with established and new therapeutic approaches.

E1358
CYCLIN D2 OVEREXPRESSION RECAPITULATES MANTLE CELL LYMPHOMA IN MICE
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Background: Mantle cell lymphoma (MCL) is a highly aggressive subtype of B-cell lymphoma that is characterized by a poor response to current treatment regimens. Most MCLs carry a prototypical translocation, t(11;14), which juxtaposes the CCND1 gene towards the immunoglobulin heavy chain (IGH) locus, resulting in cyclin D1 overexpression. Notably, a subset of MCL patients are cyclin D1 negative but instead overexpress cyclin D2 (encoded by CCND2) as a consequence of recurrent genomic rearrangements involving the CCND2 locus.

Summary/Conclusions: Our results provide evidence that STAT3 mutation and activation is mostly restricted to neutropenic CD8+ T-LGLL patients equipped with the CD16+/CD56- signature. The relationship between STAT3 activation and neutropenia FAS ligand related further supports to approach STAT3 inhibition as therapeutic strategy in symptomatic CD8+ TDLBCL+CD56- T-LGLL patients, obtaining the dual results of inducing apoptosis in leukemic LGL together with inhibition to FAS ligand mediated neutropenia.

E1357
STAT3 ACTIVATION MEDIATES CD8+/CD16+/CD56- T-LGLL NEUTROPENIA THROUGH FAS LIGAND SECRETION

Figure 1.
Aims: Here, we aim to recapitulate MCL in a mouse model of hematopoietic-specific overexpression of cyclin D2. Next, we want to use this preclinical mouse model to evaluate novel therapeutic strategies for the treatment of MCL.

Methods: To evaluate if cyclin D2 could act as a bona fide oncogene in the pathogenesis of MCL, we developed a conditional R26-driven Cdncd2 overexpression mouse model. For this, we used a targeting vector that contained a floxed stop cassette followed by the Cdncd2 gene and a EGFP/luciferase reporter, which was subsequently targeted in mESCs using recombinase-mediated cassette exchange (RMCE).

Results: The resulting R26-Cdncd2 mice were crossed to VavCre mice to enable biallelic R26-driven overexpression of Cyclin D2 in the entire hematopoietic system. Interestingly, these mice developed large lymphomas starting from 36 weeks of age (Figure 1A), with tumor cells showing characteristic MCL immunophenotype (CD19+CD5+CD23-). Of note, these malignant B-cells were monomorphic small-sized cells with slightly irregular hyperchromatic nuclei and disseminated into other organs such liver, spleen and the gastrointestinal tract (GI). Infiltrating the GI tract, the GI-infiltrating MCL-like lymphoma cells were SOX11 positive, as evaluated by IHC, suggesting that these tumors indeed reflect a murine form of MCL. Noteworthy, the MCL cells from this mouse model also contain a luciferase reporter, allowing accurate in vivo tracing of tumor cells in xenograft experiments. These xenograft experiments can be used as preclinical models, in which bioluminescence is used to asses the tumor burden and to monitor tumor regression upon drug treatment.

Summary/Conclusions: In conclusion, our preliminary data suggest that modeling cyclin D2 in mice, mimicking the elevated cyclin D2 levels of human MCL patients with translocations involving the CCND2 locus, is sufficient to form MCL.

E1359 
HDAC6 INHIBITION INCREASES CD20 LEVEL BY STIMULATING TRANSLATION OF CD20 mRNA

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Background: HDAC6 (histone deacetylase, isoform 6) is a novel promising target in hematological malignancies. HDAC6 is an atypical member of HDAC family that regulates the acetylation status, and thus the function of cytosolic proteins, and has been explored therapeutically for its role in the process of protein degradation. HDAC6 mediates the transport of protein aggregates to the autophagic machinery to diminish their cytotoxicity. Thus, the disruption of the aggresome pathway, similarly to proteasome inhibition, results in a massive accumulation of misfolded protein aggregates and apoptotic cell death. As this strategy holds a considerable potential in aggressive B-cell tumors with a high rate of protein synthesis, HDAC6 inhibitors - are currently being tested in Phase I and II clinical trials in multiple myeloma and non-Hodgkin lymphoma. The strategy holds a considerable potential in aggressive B-cell tumors with a high rate of protein synthesis, HDAC6 inhibitors are currently being tested in Phase I and II clinical trials in multiple myeloma and non-Hodgkin lymphoma. We demonstrate that HDAC6 inhibition increases translation of CD20 mRNA on polysomes without affecting general synthesis of novel proteins in the cell. However, our experiments suggest that the increased translation of CD20 mRNA is not a result of resumed translation of its transcription. Moreover, we demonstrate that HDAC6 inhibition increases translation of CD20 mRNA on polysomes without affecting general synthesis of novel proteins in the cell. However, our experiments suggest that the increased translation of CD20 mRNA is not a result of resumed translation of its transcription.

Methods: We used qRT-PCR and Dual Luciferase Assays in order to determine the influence of HDAC6 on CD20 transcription. We used pulse-chase assays using widely translated protein indicators – cycloheximide and homoharringtonine. In order to study the effect of HDAC6 inhibition on global as well as specific translation, we developed a novel synthesis of CD20 we optimized Click-IT chemistry methods. In order to study CD20 translation on polysomes we performed polysomes profiling followed by qRT-PCR. To get an insight into molecular mechanism of increased translation of CD20 after HDAC6 inhibition we studied the formation of stress granules (SG).

Results: We show that HDAC6 inhibition regulates CD20 level without affecting its transcription. Moreover, we demonstrate that HDAC6 inhibition increases translation of CD20 mRNA on polysomes without affecting general synthesis of novel proteins in the cell. However, our experiments suggest that the increased translation of CD20 mRNA is not a result of resumed translation of its transcription.

Summary/Conclusions: Our study shows a new mechanism of the regulation of CD20 expression by increasing its translation. Moreover, we demonstrate a new role of HDAC6 protein. This finding has a potential clinical application, as HDAC6 inhibitor are being widely tested in different hematological malignancies. This finding has a potential clinical application, as HDAC6 inhibitor are being widely tested in different hematological malignancies.

E1360 
CARD11 DUPLICATION AT DIAGNOSIS IDENTIFIES VERY LOW-RISK MANTLE CELL LYMPHOMA PATIENTS: RESULTS OF THE LYMA-GENOMIC PROJECT CONDUCTED ON BEHALF OF THE LYSA GROUP

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Background: Mantle cell lymphoma (MCL) is an incurable heterogeneous disease with a median overall survival (OS) of around 4-6 years. There are 3 prognostic groups of patients: a high-risk (HR) group of 15-20% of patients having a survival <1yr after diagnosis, an intermediate-risk (IG) group that includes patients remaining in response one year after EOT but with an incidence of relapse of 10-15%/yr thereafter, other patients defining the low-risk (LR) group remain in response three years at least. The MIPI score (age, leukocytosis, PS, stage) helps to classify patients according to their risk of relapse but it is not currently possible to treat patients according to risk factors.

We aim to explore if copy number alterations (CNA) detected in 96 young MCL patients treated in the LyMa trial (Le Gouill et al. Abstract 145, ASH 2016) can help to stratify patients at diagnosis into high, intermediate and low risk groups.

Methods: Samples were selected according to material availability. Lymph node biopsies collected at diagnosis, formalin-fixed and paraaffin-embedded were used to extract DNA, usable even when highly degraded since the Oncoscan® FFPE assay is optimized for highly degraded FFPE samples. Whole-genome copy number profiling was analyzed with 50 ng of genomic DNA DNA. TuScan algorithm (Affymetrix) was used to analyze data. The frequency and prognosis impact of CNAs were evaluated with univariate analysis of survival data.

Results: Characteristics of the 96 patients were as follow: median age 57y (41-65); 82% of males, MIPI-low/intermediate/high respectively 19%, 51% and 30%, blastoid morphology in 42%. No significant difference was observed between these patients and the LyMa patients (n=299). Among the 96 patients, 9 were HR patients with primary refractory disease or early relapse within one year post-diagnosis while 87 patients remained in response more than one year after diagnosis (including 64 LR patients who were still in complete remission more than 30 months after diagnosis). After ASCT, 41 patients (43%) were randomized in the rituximab maintenance arm and 40 (42%) in the observational arm. AEs follow-up from diagnosis to ASCT was median 4.8 years (range 1-14.5y).

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We assessed the mechanism of increased translation of CD20 we optimized Click-IT chemistry methods. In order to study CD20 translation on polysomes we performed polysomes profiling followed by qRT-PCR. To get an insight into molecular mechanism of increased translation of CD20 after HDAC6 inhibition we studied the formation of stress granules (SG). We also show that HDAC6 inhibition regulates CD20 level without affecting its transcription. Moreover, we demonstrate that HDAC6 inhibition increases translation of CD20 mRNA on polysomes without affecting general synthesis of novel proteins in the cell. However, our experiments suggest that the increased translation of CD20 mRNA is not a result of resumed translation of its transcription.

Summary/Conclusions: Our study shows a new mechanism of the regulation of CD20 expression by increasing its translation. Moreover, we demonstrate a new role of HDAC6 protein. This finding has a potential clinical application, as HDAC6 inhibitor are being widely tested in different hematological malignancies. Further studies in order to identify other targets for HDAC6 are required.

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Figure 1.
Summary/Conclusions: Our study confirms the worse impact of TP53 and CDKN2A deletion on early relapse in MCL. By contrast, the CARD11 duplication...
is associated with an absence of relapse and thus defines a new group of very low risk patients. These findings provide important clues for future therapeutic-driven therapies in MCL.

E1361

CLINICOBIOLOGICAL FEATURES OF B-CELL NEOPLASMS WITH CDK6 TRANSLOCATIONS: FREQUENT ASSOCIATION WITH MARGINAL-ZONE LYMPHOMA, CONTIGUOUS OF PROLYMPHOCYTIC CELLS AND TP53 ABNORMALITIES. A GFCH STUDY

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Background: Translocation involving the CDK6 gene is a rare but recurrent abnormality in B-cell neoplasms. Three different translocations have been described: t(2;7)(p11;q21), which is the most frequent, (7;14)(q21;q12) and (7;14)(q21;q11), leading to juxtaposition of CDK6 gene with IGK, IGH or IGL locus respectively.

Aims: The Groupe Francophone de Cytogenetique Hématologique (GFCH) collected 35 chronic B-cell disorders with CDK6 translocation in order to document the clinicobiological features of this uncommon aberration.

Methods: Clinical and biological data were gathered at diagnosis when available. A cytogenetical review was performed by 3 experts in 27/35 cases. FISH was used to detect IG or TRAD and CDK6 rearrangements, and recurrent abnormalities frequent in SMZL and CLL (trisomy 3, 12, 18, deletions of 7q, 11q). Immunophenotypical data were also collected.

Results: When all cases were considered, the most frequent translocation was t(2;7)(p11;q21) (22%), trisomy 3/3q (17%) and trisomy 12 (11%). Deletion of 7q and 11q were frequently observed. CDK6 was mutated in 6/22 patients (27%), including 14/19 MZL and 3/4 UBCL cases. Deletion of 7q and 11q were observed in 17/27(63%) cases, including 14/19 MZL and 3/4 UBCL cases. Our series, the CDK6+ MZL cases differed from classical SMZL by frequent prolymphocytic differentiation (14/19, 74%), very low incidence of 7q deletion (1/23, 4%), high frequency of TP53 abnormality (12/23, 52%), absence of NOTCH2 mutation (0/13, 0%), and a different IGHV repertoire with low frequency of VH1-2 (1/11). The CDK6+ UBCL also had frequently a contingent of prolymphocytes (3/4, 75%), and showed a genetic profile similar to the CDK6+ MZL (see figure).

Summary/Conclusions: These results, obtained on the largest series to date, suggest that CDK6 translocation is associated with indolent small B-cell lymphomas, mostly SMZL, with distinctive features. However, CDK6 translocations were infrequently observed in preliminary studies on small cohorts. We describe one case involving the T-cell receptor (TCR) locus, which is a rare event in B neoplasms. Finally, it is intriguing that this abnormality involves almost exclusively the IGK locus, but not the other Ig loci, especially IGH which is usually the most frequently rearranged.

E1362

PRIMARY CUTANEOUS DIFFUSE LARGE B-CELL LYMPHOMA, LEG TYPE, EXPRESS STEREOTYPED B-CELL RECEPTORS WITH UNIQUE NONSYNONYMOUSLY MUTATED CONSTANT REGIONS

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Background: Primary Cutaneous Diffuse Large B-Cell Lymphoma, Leg Type (DLBCL, LT) is a rare and aggressive neoplasm with a primary cutaneous presentation that shares genetic and phenotypic characteristics with DLBCL of activated B-cell subtype (ABC-DLBCL). Although receptor stereotypes have been observed, the role of the B-cell receptor (BCR) in DLBCL, LT is largely unknown. Previous studies on small cohorts suggested that DLBCL, LT expresses IgM with overrepresentation of IGHV3 alleles and high rates of somatic mutations.

Aims: We aimed to elucidate the stereotype of the BCR in DLBCL, LT and to test for autonomous antigen-independent signalling as described for CLL (Dührren-von Minden, Nature 2012) and non-leg-type ABC-DLBCL (Koning, AACR 2016 & ASH 2016).

Methods: 8 cases of DLBCL, LT were subjected to RNAseq. Additional RNAseq data from 6 healthy volunteers (GEUVADIS project), 10 non leg type DLBCL, and 16 follicular lymphoma were obtained from NCBI publicly available databases and collaborators. VDJ/VJ rearrangements and IgM constant regions were Sanger sequenced for all cases and two granulocyte controls. Lymphoma-derived, clonal BCR were tested for autonomous signalling activity in the murine TKO pre-B-cell system (Dührren-von Minden, Nature 2012).

Results: RNAseq analysis demonstrated an IgM isotype in all eight and VJ-kappa rearrangements in seven of DLBCL, LT cases. V(D)J BCR of DLBCL, LT on a murine constant region backbone did not induce antigen-independent calcium flux in TKO cells upon induction of functional activation of the BCR signalling cascade by tamoxifen.

Summary/Conclusions: Our data identify a clearly stereotyped receptor in DLBCL, LT. In contrast to CLL and ABC-DLBCL, BCR stereotypy was not associated with autonomous BCR signalling activity using a murine IgM backbone. The pathogenic potential of the novel constant region mutations for BCR activity in DLBCL, LT warrants further functional studies.

E1363

LOSS OF NR4A1 ACCELERATES MYC-DRIVEN LYMPHOMAGENESIS ACCOMPANYED BY OVEREXPRESSION OF GENES INVOLVED IN IMMUNOREGULATION

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Background: NR4A1 (Nurr77) belongs together with NR4A2 (Nurr1) and NR4A3 (NOR-1) to the Nurr77 family of nuclear orphan receptors. As immediate early- or stress response genes their expression is diverse as it is the cellular outcome upon activation. Recently, there has been attributed a pivotal role to NR4A1 and NR4A3 as tumor suppressors in AML in humans and mice. In our comprehensive NR4A4 expression analysis in various lymphoma entities we demonstrated a significant reduction of NR4A1 expression in aggressive lymphomas, which was associated with poor cancer-specific survival. Moreover, ectopic expression of NR4A1 in aggressive lymphoma cells resulted in induction of apoptosis.

Aims: In order to better dissect the role of NR4A1 in lymphoid malignancies, we used a Myc-driven mouse model of lymphomagenesis and crossed the EµMyc mouse with the Nr4a1-/- mouse. Survival and tumor formation were monitored and RQ-PCR was performed on selected tumor specimens, whereby genes, found to be associated with NR4A1 expression in the publicly available gene expression data set of DLBCLs generated by Lenz et al., were taken. Moreover, the driver-function of NR4A1 in lymphomagenesis at the premalignant stage was investigated by using apoptotic assays and by carrying out transplantations of tumor cells into wt recipients.

Methods: Kaplan Meier analysis was performed for survival and tumor formation in EµMyc Nr4a1+/+ (n=154), EµMyc Nr4a1+/- (n=54) and EµMyc Nr4a1-/- (n=59), respectively. For RQ-PCR selected tumor specimens from wt and EµMyc mice with (n=14) and without (n=17) Nr4a1 loss were used. For investigation of the role of Nr4a1 at the premalignant stage, mice aged 4 weeks (n=41 per genotype) were sacrificed and AnnexinV staining and cleaved-caspase3 assay were performed on cells isolated from the spleen and bone marrow. In vivo expression of Nr4a1 driven by its own promoter was induced by tumor cells from Nr4a1-/- mice injected into the tail vein of wt mice. Kaplan Meier analysis was used for monitoring survival and tumor formation, and FACS analysis for analysis of bone marrow, spleen and tumor, respectively.

Results: EµMyc Nr4a1+/- mice showed decreased survival with a median of 92 days for EµMyc Nr4a1+/- with median survival of 123 days (p=0.001) and tumors developed faster with a median of 45 days for EµMyc Nr4a1+/-, vs 107 days for EµMyc Nr4a1+/-; p<0.001. Both, survival (median=101 days; p=0.037) and tumor formation (median=66 days, p=0.001) gave intermediate values for EµMyc Nr4a1+/- mice. Furthermore, EµMyc Nr4a1+/- expression was associated with a significantly increased cell subpopulation at the premalignant stage, whereas apoptosis was significantly diminished in EµMyc Nr4a1-/- mice. RQ-PCR showed that several genes involved in immunoregulation and Nr4a1 target genes were upregulated in EµMyc Nr4a1+/- compared to EµMyc Nr4a1+/-; p<0.001. Last, tumor formation upon i.v. injection that tumors in Nr4a1-/- mice were engrafted faster than tumors derived from mice without Nr4a1 loss (25 days vs 38 days; p=0.009) and lead to a decreased number of inflammatory cells in the tumor.

Summary/Conclusions: Our results clearly demonstrate the influence of Nr4a1 loss on tumor formation and consequently survival in a Myc-driven model of lymphomagenesis. Importantly, Nr4a1 loss seems to impact cell death early in B cell development, even ahead of malignant transformation. Additionally, Nr4a1 seems to be involved in driving immune responses towards an anti-inflammatory, tolerogenic phenotype, thereby facilitating tumor growth and in altering the tumor environment. Collectively, these data underpin the tumor suppressive function of Nr4a1 in aggressive lymphomas.

E1364

DISSECTING THE PI3K PATHWAY IN A CYCLIN D1-DRIVEN MODEL OF MCL

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Background: Mcl cell lymphoma (MCL) presents as a highly disseminated B-Cell malignancy, accounting for about 6% of all non-Hodgkin lymphomas. Genetically, MCL is characterized by the t(11;14)(q13;32) translocation, leading to the overexpression of the cell cycle regulator Cyclin D1. The disease is associated with poor survival and can be treated with targeted therapies. Therefore, it is important to define new therapeutic strategies. Interestingly, the PI3K/mTOR pathway has emerged as a promising therapeutic target in MCL, as cell lines and patients have shown substantial response rates to rapamycin and analogs.

Aims: The aim of this study is to functionally dissect the role of individual PI3K and PI3K-pathway genes by performing a shRNA-based screen in genetically defined primary murine MCL tumor cells. Hereby, we want to identify synthetic lethal genes for Cyclin D1 and novel molecular dependencies in Cyclin D1-driven lymphomagenesis, thereby establishing novel potential therapeutic targets in MCL.

Methods: We have developed a new mouse model for MCL using Eµ-myc transgene mice that overexpress the MCL hallmark lesion Cyclin D1, as well as the reverse tet transactivator for inducible transgene expression. Using primary MCL tumor cell lines derived from this model as a platform, we performed shRNA loss-of-function screen entailing a two colored, antibiotic selectable and tel-inducible retroviral shRNA expression vector system. A shRNA library targeting more than 300 different PI3K related genes was introduced into primary murine MCL cells. After induction of shRNA expression by addition of Dox, shRNA representation in knockdown and control cells was deconvoluted by deep sequencing to identify differentially selected shRNAs. The shRNA screen identified more than 70 strongly (> 4 fold) differentially regulated genes affecting MCL tumor growth and survival. We identified numerous targets within the PI3K pathway and the molecular dependency on this pathway was in line with the observed high sensitivity of these cells towards pharmacological mTOR inhibitor. Individual shRNA knockdown experiments confirmed the newly identified candidate genes including components of the lipid second messenger system, such as diacylglycerol kinase isoform alpha (Dgka) and gamma (Dgkg). Knockdown of these lipid kinases by three or two different hairpins lead to decreased cell proliferation. Dgka knockdown was further validated on protein level by Western Blot analysis. Furthermore, these newly identified candidate genes will be further explored to characterize their role in Cyclin D1-driven lymphomagenesis, with the aim of identifying novel therapeutic targets in this difficult-to-treat disease.

E1365

MUTATIONAL PROFILING OF HODGKIN- AND REED-STERNBERG CELLS (HRSC) OF CLASSICAL HODGKIN LYMPHOMA (CHL) ENRICHED FROM ARCHIVAL FORMALIN-FIXED AND PARAFFIN-EMBEDDED TISSUE SAMPLES

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Background: CHL can be cured in the majority of cases. However, ~10–20% patients die of the lymphoma after relapse or progressive disease. There are unmet needs for understanding the mechanisms that cause CHL relapses, for development of new prognostic/predictive markers and effective targeted therapies. Comprehensive genetic characterization and advance in understanding the molecular pathology of CHL are indispensable to meet those needs. However, genetic information on CHL is still scarce mainly due to difficulties of isolating malignant HRSC, whose overall frequencies in the affected tissues range from 0.1-5%. Formalin-fixed paraffin-embedded (FFPE) tissue archives are the most abundant source of clinically annotated tumor specimens. However, FFPE tissue quality is limited because of poor DNA quality and difficulty to enrich neoplastic cells. Therefore, new enrichment techniques are necessary to enable large scale comprehensive genetic investigations of CHL.

Aims: Our aims were: 1) to develop a technique for HRSC enrichment from the archival formalin-fixed paraffin embedded tissue; 2) to reliably detect genetic aberrations in the genomes of enriched tumor samples and to use this information for development of new prognostic and predictive markers as well as for better understanding of the genetic background of CHL.

Methods: We have developed a new high-throughput method for marker-based enrichment of archival FFPE tissue-derived HRSC nuclei by fluorescence-assisted cell sorting (FACS). Genomic DNA extracted from sorted nuclei was used for identification of mutations in 68 genes that are frequently mutated in lymphomas by targeted high throughput sequencing (HTS). Chromosomal copy number aberrations were investigated by the Agilent SurePrint 180k microarray.

Results: Enzymatically extracted FFPE tissue-derived cell nuclei retain their genome integrity and can be used with multiplexed antibodies against nuclear (MUM1, PAX5) and cytoplasmic/cell surface (CD30) markers. Chromatin copy number aberrations were detected for 107 genes with a median of 6.67 chromosomal regions per sample. Taken together our study demonstrates that DNA extracted from the enriched cell populations is suitable for large-scale comprehensive genetic profiling of CHL.

Summary/Conclusions: A novel rare-cell-enrichment technique is suitable for genetic CHL studies and opens the possibility for the wider use of archived
E1366
LACK OF STAT1 PREDISPOSES TO A DIFFUSE LARGE B-CELL LYMPHOMA-LIKE DISEASE
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Background: The highly conserved JAK-STAT signaling pathway regulates proliferation, differentiation, apoptosis and immune responses. Activating mutations in STAT3 are considered to drive the development of diffuse large B-cell lymphomas (DLBCL). STAT1 is a critical counter player of STAT3. Of note, many STAT1 target genes are frequently altered or mutated in DLBCL patients, such as SOCS-1, B2M, PDL1, CARD11, CIITA and BCL6. We observed that the loss of STAT1 suffices to provoke spontaneous haematopoietic tumors in mice.

Aims: We aimed at investigating the underlying mechanisms of spontaneous hematopoietic tumor formation in STAT1-deficient mice.

Methods: We characterized the spontaneous haematopoietic tumors by FACS and morphological analysis. To identify the cell of origin for the disease, we performed bone marrow transplantation assays. We high-purity FACS-sorted individual cell populations of diseased STAT1-deficient mice and transplanted them into recipient mice. Ex vivo RNA sequencing was performed on both the malignant B-cell population and the non-malignant control. We performed bone marrow transplantation assays. We high-purity FACS-sorted individual cell populations of diseased STAT1-deficient mice and transplanted them into recipient mice. Ex vivo RNA sequencing was performed on both the malignant B-cell population and the non-malignant control.

Results: STAT1-deficient mice develop a myeloid hyperplasia that manifests with an incidence of 60% and is characterized by the absence of Rigi. Transplantation of bone marrow unmasked the development of a B-cell malignancy, which can be transferred by CD19+ cells. The malignant B-cells arising in STAT1-/ mice can be maintained in vitro and display alterations in gene expression that are typical in human DLBCL such as Irf4, Prdm1 and p53. RNA-seq analysis revealed features shared with human DLBCL: increased reads a locus of B, Met2h, Card11 and Cd274 (PDL1) and decreased expression of Socs-1, Cdkn2a, B2m and Prdm1. Low levels of STAT1 combined with low levels of p16INK4A correlate with a reduced life expectancy in DLBCL patients.

Summary/Conclusions: Loss of STAT1 in B cells-mice provokes a myeloid hyperplasia which masks a B-cell malignancy resembling human DLBCL. DLBCL patients with low levels of STAT1 have a poorer prognosis if they lack the tumor suppressor p16INK4A.

E1367
MOLECULAR HETEROGENEITY OF MANTLE CELL LYMPHOMA
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Background: Mantle cell lymphoma (MCL) is a B-cell non-Hodgkin lymphoma characterized by (t11;14)(q13;q23) leading to constitutive cyclin D1 overexpression and cell cycle deregulation. The survival is still poor, especially for patients resistant to frontline drugs. Although patients are brought in remission, relapses often occur with disseminated lymphoma, which is more difficult to treat. There is a need for a better understanding of the clonal heterogeneity of this disease and to identify new signaling pathways with genes which could be targeted by novel drugs or be used as biomarkers to predict response to treatment.

Aims: To address the genetic heterogeneity in MCL in paired patient samples at diagnosis and relapse.

Methods: Highly pure malignant B-cell populations were isolated using fluorescence-activated cell sorting in four patients diagnosed with MCL. In addition T-cells were sorted from the same patients as paired non-malignant control samples. RNA was performed on both the malignant B-cell population and paired T-cells (13 samples in total). Mutations were detected in parallel with CLC Biomedical Workbench 2.5 (Qiagen) and MuTect 1.04 (Broad Institute) (coverage > 20, population allele frequency<0.01) and evaluated against the COSMIC (Wellcome Trust Sanger Institute), dbSNP and PubMed databases. Exonction from informed consent was approved by the National Ethical Committee.

Results: Our data highlighted in each patient persistent gene modifications between diagnosis and relapse. We confirmed gene mutations already well-known in B-cell malignancies (e.g. TP53, NOTCH1 and MYD88). Interestingly, aberrations not previously described in the COSMIC database, were observed with high allele frequency both at diagnosis and at relapse. This included genes in B-cell signaling (e.g. transcriptional repressor SPEN associated to NOTCH pathway regulation and blockage of the precursor B-cell differentiation), inflammatory response (e.g. IRG1), genes found in invasive carcinoma (e.g. integrin β4 subunit) and B-PLA2. These modifications might be caused by somatic mutations or hit in putative drivers, new gene modifications as well as loss of previous ones could be observed at relapse. For example, genes involved in embryonic development and cell fate (e.g. the transcription factor SOX1) and genes involved in inflammation (CCL13) were not previously correlated to MCL and were novel at relapse. This suggests that a modified malignant clone has evolved and progressed. No gene modification was observed by all four patients. However, aberrations in the same signaling pathways were identified across individuals. From allele frequency distribution detected with MuTect we could detect discrete clonal or competing subclonal involvement: A patient harbored one major discrete clone at diagnosis while at relapse two clones were observed, whereas in other patient presented a diffuse clonal pattern at diagnosis and a more discrete biconal pattern at relapse.

Summary/Conclusions: Our work shows examples of molecular progression from diagnosis to relapse in MCL and supports the heterogenic nature and genetic complexity of this disease. We confirm mutations in genes already known as involved in the biology of MCL and we identified new ones involved in the B-cell signaling pathways. This adds valuable knowledge to the biological understanding of MCL which is pivotal in the era of precision medicine.

E1368
NOVEL TARGET GENES OF DEREGULATED MIRNAS IN DLBCL REVEALED BY ENDOGENOUS AGO2 PAR-CLIP
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Background: Aberrant expression of microRNAs (miRNAs) is a widespread phenomenon in cancer. However, the functional role of this population is poorly understood as the target genes of miRNAs (the targetome) are notoriously difficult to predict computationally and moreover differ according to cellular context. An alternative approach is to directly sample the targetome using immunoprecipitation (IP) techniques such as PAR-CLIP. The drawback however of such techniques is the need for exogenously produced tagged protein complexes. In order to identify large B-cell lymphomas (DLBCL) as a common form of non-Hodgkin lymphoma, typically aberrantly expressing miRNA-155 (Lawrie, 2007). This miRNA is a well known key regulator of lymphomagenesis, needed for T and B cell function (Rodriguez, 2007; Thal, 2007; Vigorito, 2007), and its over-expression in pre-B cells or haematopoietic stem cells leads to oncogenic transformation (Costinean, 2006; O’Connell, 2008). Given their relevance, DLBCL and miR-155 were chosen as models for the present study.

Aims: We set ourselves to adapt PAR-CLIP technology to allow non-engineered cells to be used based on IP of endogenous levels of Ago2. In addition, we also aimed at testing the minimum number of input cells needed for miRNA target identification.

Methods: Two DLBCL cell lines (ABC-type RIVA, and GC-type SUD-HL10) were transfected with lentiviral vectors that encoded miR-155. In parallel, we transfected these cells with an inhibitor of miR-155 or with a scrambled sequence, as experimental controls (for reducing the number of false positives). Cells were then stably selected with puromycin, and grown in the presence of 100 μM 4SU for 18 h. Different amounts of these cells (300M, 50M and 10M) were then irradiated to cross-link the RNA to RNA-binding proteins. PAR-CLIP was then performed on cell lysates using anti-Ago2 mAbs for IP. The original protocol (Hafner, 2010) was modified to eliminate radioactive labelling. The reverse transcriptase was used for library building using TruSeq Small RNA Sample Kit v1 and the sequencing performed on an Illumina HiScanSQ system. After deduplication and alignment, T-to-C variants (indicative of miRNA-dependent target identification).

Results: Endogenous Ago2 IP, followed by a radioactive-free modified PAR-CLIP protocol yielded sufficient RNA for building libraries for NGS irrespectively of cell input. Samples gave an average ~9.7 x10⁶ aligned reads/library. There were an average of 3,730 PAR-CLIP clusters mapping to coding genes (range 4,675 - 11,004, correlating with the number of input cells, r=0.82). In all exper-
imential conditions we found that a number of the captured genes corresponded to experimentally validated targets of miR-155. Crucially, ontogeny analysis of the PAR-CLIP-captured genes demonstrated an enrichment of genes involved in haematopoietic and/or lymphomagenesis pathways. Summary/Conclusions: To fully understand the role of a particular miRNA in the specific malignancy, it is essential to identify its target genes in a relevant cellular context. Using a haematopoietic malignancy model of high clinical interest we have developed an optimised method for interrogating the miRNA:mRNA interface (targetome) within a cellular system without the need of ectopically expressed Ago2, keeping physiological levels of the core component of the RISC complex unaffected. Moreover, our optimized protocol allowed us to reduce the number of input cells, therefore opening the exciting possibility of interrogating the targetome of patient primary samples.

E1369 DARATUMUMAB, A NOVEL HUMAN CD38 MONOCLONAL ANTIBODY FOR THE TREATMENT OF B-CELL NON-HODGKIN LYMPHOMA A. Matas-Céspedes1, A. Vidal-Crespo1, V. Rodriguez1, C. Rossí2, G. Roué1, A. López-Guillermo3, E. Giné3, A. Wiestner4, C. Bezomoses2, E. Campo5, D. Colomer5, S. Balasubramanian6, C. Chiu6, P. Doshi6, P. Pérez-Galán1

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Background: Daratumumab (DARA) is a humanised monoclonal antibody that targets the CD38 epitope and is approved for the treatment of relapsed/refractory (R/R) multiple myeloma (MM) patients. DARA is currently being evaluated in phase II clinical trials as monotherapy in patients with R/R Mantle Cell Lymphoma (MCL), Follicular Lymphoma (FL) and Diffuse Large B-Cell Lymphoma (DLBCL). In vitro and in vivo experiments show that DARA induces cell death through high-affinity mediated mechanisms in MM, including Antibody-Dependent Cellular Cytotoxicity (ADCC), Complement-Dependent Cytotoxicity (CDC) (de Weers M. J Immunol, 2011) and Antibody-Dependent Cellular Phagocytosis (ADCP) (Overdijk MB. MAbs, 2015). In Chronic Lymphocytic Leukemia (PLL), DARA induces killing mainly via ADCC and ADCP (Matas-Céspedes A. Clin Cancer Res, 2016). Furthermore, Immunomodulatory effects (Krijnck J. Blood, 2016) and modulation of the enzymatic activity of CD38 (Lamerts van Bueren J. Blood, 2014) have been described to contribute to its antitumor activity. Aims: To evaluate the activity of DARA on MCL and FL cells as monotherapy and in combination with current therapies, both in vitro and in vivo. Methods: ADCC, CDC and ADCP activities were assessed by calcein release or flow cytometry. Penetration of DARA was analyzed in a 3D model by Selective Plane Illumination Microscopy (SPIM). Molecules per cell were analyzed using Qifikit and flow cytometry. In vivo activity was assessed in prophylactic and therapeutic set ups using SCID mice subcutaneously (sc) or intravenously (iv) injected with 1x10⁶ of MCL or FL cells. Mice were treated (human IgG control or DARA) with two different schedules: prophylactic (3 doses of 10mg/kg one dose per week) or therapeutic (20/10/10/10mg/kg, one dose per week). For the combination regimens in FL, sc injected SCID mice were treated following the combination schedule in combination with Rituximab (20/10/10/10mg/kg, one dose per week) and/or CHOP (initial unique dose). Results: DARA (0.0001-1µg/mL) induced ADCC in a dose-response manner on MCL (n=6) and FL (n=4) cell lines in the presence of PBMCs in vitro. Moreover, DARA induced significant levels of ADCP at 1µg/mL on MCL (n=6) and FL (n=4) cell lines in the presence of human macrophages in vitro. However, DARA did not induce significant CDC in any of these models due to a high expression of the complement inhibitors CD46, CD55 and CD59, and insufficient number of CD38 molecules per cell. In a 3D model of FL, SPIM analysis revealed a maximum penetration of DARA at 1µg/mL after 48h of treatment. We tested DARA activity in vivo in two different mouse models (sc and iv) of MCL and FL. In the prophylactic setting, DARA completely prevented the outgrowth and induced tumor regression of MCL (n=6) and FL (n=6) subcutaneous tumors. In the therapeutic setting, DARA significantly increased the overall survival of mice in both models of tumor cells both in the MCL (n=10) and in the FL (n=10) systemic xenograft models. In addition, the combination of DARA with Rituximab/CHOP regimen in FL, resulted in a synergistic reduction of tumor growth (n=7-10). Summary/Conclusions: DARA shows encouraging cytotoxic activity in MCL and FL cells in vitro and in vivo. In addition, DARA exerts unique and substantial effects as single agent on MCL and FL tumor cell growth in different mouse models and contributes to potent therapeutic efficacy in combination with current approved therapies. These results warrant further studies of DARA in the clinical setting for these conditions.

E1370 ECTONUCLEOTIDASES CD39/CD73 ARE HIGHLY EXPRESSED ON ATLL CELLS AND RESPONSIBLE FOR GENERATING AMP/ADENOSINE Y. Nagate1, S. Ezeo1, J. Fujita1, M. Ichi1, J. Toda1, K. Oritani1, Y. Kanakura1

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Background: Adult T-cell leukemia/lymphoma (ATLL) is a mature T-cell neoplasm, linked to the human T-cell lymphotropic virus, HTLV-1. Patients with ATLL are often at the risk of opportunistic infections. It might be possible that the immunocompromised state could be induced by the function of ATLL cells having similar phenotypes with regulatory T cells (Tregs). However, difficulties of in vitro studies using primary tumor cells have hampered the progress of ATLL research, and it is still controversial whether ATLL tumor cells have the immunosuppressive characteristics. Aims: In this study, we analyzed the roles of molecules expressed in ATLL cells associated with immunosuppressive functions of Tregs. Methods: The protocol of this study was approved by the Investigational Review Board of Osaka University Hospital. Peripheral blood mononuclear cells (PBMCs) were collected from 8 asymptomatic HTLV-1 carriers and 20 ATLL patients (3 with smoldering type, 5 with chronic type, and 12 with acute type) after getting informed consent. PBMCs from 3 ATLL patients were separated into CD4+CD7-CD3+CD1+ T cells and adjacent CD4+ CD7+CD3+ normal T cells using Fluorescence-activated Cell Sorter (FACS), and total RNA sequencing experiments were conducted. And we also examined the expression patterns of CD39 and CD73 in ATLL patients. Results: We compared whole transcriptome of ATLL cells and normal CD4+ cells. Bioinformative analyses showed that many genes associated with immunosuppressive functions of Tregs were elevated or downregulated in ATLL cells. Among these we focused on CD39, CD73 and CD26, because recently it has been reported that extracellular adenine, which is catalyzed by CD39, expressed in human Tregs, and CD73, expressed in murine but not in human Tregs, has strong anti-inflammatory function and plays major role in Treg-mediated immunosuppression. Therefore, we investigated the expression of CD39 and CD73 in ATLL cell lines and primary tumor cells. We found that all of 4 ATLL cell lines expressed CD39, but not CD73 as human effector Tregs. In contrast, the expression patterns of CD39 in 20 ATLL patients were various (Table) and interestingly, some ATLL tumor cells express CD73. Also in asymptomatic carriers, we could detect CD39 and/or CD73 positive on CD3+CD4+CD1+CD7 negative cells in human naive but not in effector Tregs, was negative in all cell lines and primary cells except for abnormal cells in one smoldering patient. Next, the role of CD39 and/or CD73 in ATLL cells was assessed. Extracellular ATP is converted into AMP by CD39. As expected, CD39+ ATLL cells converted significantly more ATP than CD39+ ATL cells, which were comparable with normal effector Tregs. Conversely, mass spectrometry analysis of AMP/adenosine concentration indicated the activity of CD39 mediated AMP hydrolysis was very slow; less than 10% of 1mM AMP was converted to adenosine by CD73+ ATL cells, indicating that the aberrant expression of CD73 could not efficiently increase adenosine synthesis.

Summary/Conclusions: In this study, we showed that about two thirds of ATLL samples were CD39+CD26+ just as effector Tregs and have comparable level of ATPase activity as Tregs, which are expected to play some immunosuppressive function in ATL patients. Recently it is also reported that in exhausted CD8+ T cells in cancer patients, CD39 is co-expressed with PD-1. CD39 expression in ATLL cells may also have some roles in immunosuppression and thus in the escape from anti-tumor immunity.
and proliferation of several B cell malignancies. BTK is a key regulator of this pathway. In a preliminary clinical study, STRO-001 showed therapeutic activity in relapsed/refractory DLBCL of the Activated B-cell phenotype (ABC-DLBCL) (Walter et al Blood 127pp411-419,2016). However, median treatment duration in ABC-DLBCL was only 3 months due to progressive disease and development of resistance. Two acquired resistant mutations in p53 and Rb66W50H have been reported as dominant negative mechanisms to BTK inhibition in CLL but resistance mechanisms in DLBCL have not been fully elucidated.

Aims: To determine resistance mechanisms in the ABC-DLBCL TMD8 cell line and determine new rational combinations to take into the clinic with ONO/GS-4059. Methods: The BTK inhibitor sensitive ABC-DLBCL cell line TMD8 was clonal ONO/GS-4059 and Ibrutinib resistant TMD8 cell lines (TMD8RO and TMD8RI) were used for this study. TMD8RO has PLCγ2 R665W whilst TMD8RI lacks both BTK C481S and PLCγ2 R665W. Cell viability and apoptosis after compound treatment were assessed using Cell titer Glo assay and Annexin V/PI staining. Immunoblotting was performed to detect downstream expression of immunoreceptor were assessed by immunoblot and Flow cytometry. The mutational status of BTK and PLCγ2 in TMD8 was determined by Sanger sequencing.

Results: ONO/GS-4059 induced apoptosis in TMD8 at nanomolar concentrations. BTK C481S and PLCγ2 R665W induced classical apoptosis in >80% of cells. Although ONO/GS-4059 induced rapid reduction in ERK and AKT activation, activation of ERK and AKT rebounded within 24 hours in surviving cells. Interestingly, surface immunoglobulin M (sigM) expression was increased more than three times in these cells leading to subsequent activation of SYK. The specific SYK inhibitor GS-9973 combined with ONO/GS-4059 inhibited the downstream ERK and AKT reactivation and induced synergistic apoptosis in TMD8. On the other hand, SYK hyper-activation as determined by phosphorylation of SYK and its downstream target BLNK was also observed in the two BTK inhibitor resistant cell lines. Additionally, expression of CDS and CD22, which negatively regulate BCR signaling, was decreased in these cells. The combination of ONO/GS-4059 and GS-9973 restored sensitivity to ONO/GS-4059 and induced synergistic apoptosis in both resistance cell lines.

Summary/Conclusions: These data show that SYK is highly activated through increased sigM expression and/or downregulated CDS and CD22 following BTK/BCR treatment. The changes may contribute not only the development but also the maintenance of resistance to BTK inhibitor. The combination of ONO/GS-4059 with SYK inhibitor is therefore a rational strategy for preventing and overcoming BTK inhibitor resistances.

E1373

STRO-001, A NOVEL ANTI-CD74 ANTIBODY DRUG CONJUGATE (ADC) FOR TREATMENT OF B-CELL NON-HODGKIN’S LYMPHOMA (NHL)

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Background: CD74 is a type II transmembrane glycoprotein involved in the formation and transport of MHC class II protein. CD74 is rapidly internalized and highly expressed in many B-cell malignancies with limited expression in normal tissues (Stein R. et al., CCR 2007). STRO-001 is a novel CD74-targeting ADC which consists of a p-azido-methyl-phenylalanine (pMFA)-containing anti-CD74 aglycosylated human IgG1 antibody (SP7219) conjugated to a non-cleavable dibenzocyclooctyne (DBCO)-maytansinoid linker-warhead. Highly efficient site-specific conjugation enabled by Sutro’s cell-free antibody production and click chemistry produced a well-defined homogeneous ADC with efficient site-specific conjugation enabled by Sutro’s cell-free antibody production and click chemistry produced a well-defined homogeneous ADC with a drug-to-antibody ratio (DAR) of 2. Due to its limited cell permeability, the major catabolite released by STRO-001 has 100X lower cell killing activity on CD74 positive and negative cells compared to a reference cytotoxic maytansinoid.

Aims: The aim of this study was to investigate the therapeutic potential of STRO-001 in non-Hodgkin’s lymphoma (NHL) cell lines and xenografts. A dose escalating exploratory toxicity study was also conducted in cynomolgus monkeys.

Methods: Biotinylated SP7219 was used for immunohistochemistry (IHC). DBCO-Alexa647-conjugated SP7219 and flow cytometry were used for detection and quantification of CD74 expression on NHL cell lines and B-cells from normal human donors. STRO-001 was used to determine the EC50 and percent span of killing in NHL cell lines. The anti-tumor activity of STRO-001 in SCID mice bearing NHL xenografts was reported as dominant negative mechanisms to BTK inhibition in B cells.

Results: Expression of CD74 in different lymphoma subtypes was evaluated by antibody-binding capacity, does not correlate with expression and proliferation of several B cell malignancies. BTK is a key regulator of this pathway. In a preliminary clinical study, STRO-001 showed therapeutic activity in relapsed/refractory DLBCL of the Activated B-cell phenotype (ABC-DLBCL) (Walter et al Blood 127pp411-419,2016). However, median treatment duration in ABC-DLBCL was only 3 months due to progressive disease and development of resistance. Two acquired resistant mutations in p53 and Rb66W50H have been reported as dominant negative mechanisms to BTK inhibition in CLL but resistance mechanisms in DLBCL have not been fully elucidated.

Aims: To determine resistance mechanisms in the ABC-DLBCL TMD8 cell line and determine new rational combinations to take into the clinic with ONO/GS-4059. Methods: The BTK inhibitor sensitive ABC-DLBCL cell line TMD8 was clonal ONO/GS-4059 and Ibrutinib resistant TMD8 cell lines (TMD8RO and TMD8RI) were used for this study. TMD8RO has PLCγ2 R665W whilst TMD8RI lacks both BTK C481S and PLCγ2 R665W. Cell viability and apoptosis after compound treatment were assessed using Cell titer Glo assay and Annexin V/PI staining. Immunoblotting was performed to detect downstream expression of immunoreceptor were assessed by immunoblot and Flow cytometry. The mutational status of BTK and PLCγ2 in TMD8 was determined by Sanger sequencing.

Results: ONO/GS-4059 induced apoptosis in TMD8 at nanomolar concentrations. BTK C481S and PLCγ2 R665W induced classical apoptosis in >80% of cells. Although ONO/GS-4059 induced rapid reduction in ERK and AKT activation, activation of ERK and AKT rebounded within 24 hours in surviving cells. Interestingly, surface immunoglobulin M (sigM) expression was increased more than three times in these cells leading to subsequent activation of SYK. The specific SYK inhibitor GS-9973 combined with ONO/GS-4059 inhibited the downstream ERK and AKT reactivation and induced synergistic apoptosis in TMD8. On the other hand, SYK hyper-activation as determined by phosphorylation of SYK and its downstream target BLNK was also observed in the two BTK inhibitor resistant cell lines. Additionally, expression of CDS and CD22, which negatively regulate BCR signaling, was decreased in these cells. The combination of ONO/GS-4059 and GS-9973 restored sensitivity to ONO/GS-4059 and induced synergistic apoptosis in both resistance cell lines.

Summary/Conclusions: These data show that SYK is highly activated through increased sigM expression and/or downregulated CDS and CD22 following BTK/BCR treatment. The changes may contribute not only the development but also the maintenance of resistance to BTK inhibitor. The combination of ONO/GS-4059 with SYK inhibitor is therefore a rational strategy for preventing and overcoming BTK inhibitor resistances.

E1373

DETECTING MALIGNANT B-CELLS IN CEREBROSPINAL FLUID: DOES THE IDEAL METHOD EXIST?

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Background: Leptomeningeal dissemination (LD) is a relatively rare but often fatal complication of lymphomas, confirmed by the analysis of the cerebrospinal fluid (CSF). The diagnosis is suspected in case of neurological symptoms, parenchymal brain involvement detected with neuroimaging techniques and lymphomatous interstitial fluid. The analysis of CSF is considered as the “gold standard” but remains insufficiently sensitive.

Aims: The aim of our study was to assess the benefit of more sensitive techniques, i.e. immunophenotyping by flow cytometry (FCM) and clonality by PCR, in the detection of LD cells in the CSF of patients with suspected leptomeningeal dissemination.

Methods: This study was conducted on 326 CSF samples (236 patients) referred to our laboratory between January 2015 and December 2016. CM, FCM and PCR results were recorded and classified as positive (+), negative (-) or classified as non conclusive (NC). Cytomorphological examination (CM) is still considered as the “gold standard” but remains insufficiently sensitive.

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formed following the BIOMED-2 design and protocol. All PCR experiments were done in duplicates, and cases were considered PCR+ when both duplicates showed the same clonal pattern, ruling out false positivity (pseudoclonal pattern) often seen in paucicellular samples.

Results: We confirm that FCM and PCR are more sensitive than CM. Indeed, every CM+ cases (n=16) was also FCM+ and/or PCR+ while 13 cases were FCM+/PCR+ but CM-. A total of 269 samples showed similar results by FCM and PCR with presence (n=22) or absence (n=247) of lymphomatous cells whereas 25 samples were classified as suspicious by at least one technique. Eleven samples were FCM+ but PCR-. False negative (FN) PCR results can be explained in part by extensive somatic mutation in IG genes, preventing optimization of the primers and/or antibody staining. However, the levels of BCL-2 targets less prone to somatic mutations, such as IGL, should therefore be evaluated. Conversely, 21 samples were PCR+ but FCM-. Absence of FCM detection might have resulted from the presence of very large lymphomatous cells outside the scope of analysis. Also, rapid cell death is an issue with FCM (preventing the use of intracellular staining). Moreover, molecular techniques do not systematically require intact cells. Most of the difficulties encountered with both methods are due to occult blood contamination and poor cellularity, leading to low-intensity clonal signals by PCR and inconsistent clustering of events with FCM. In addition discordant results between FCM and PCR might be explained by sampling heterogeneity. Considering these limitations, it seems highly advisable to choose the best suited method for the follow-up according to the results at diagnosis.

Summary/Conclusions: Our results suggest that a multimodal investigation using FCM and PCR is necessary for improved detection of leptomeningeal dissemination in B-cell malignancies. It seems premature to make clinical decisions based on a single technology. Both methods, which suffer limitations that need to be acknowledged, are complementary and should be performed at diagnosis. Specific limitations of each of them should be taken in consideration for follow-up studies.

E1376

THE SYK INHIBITOR R406 DRAMATICALLY INCREASES THE SENSITIVITY OF GCB AND ABC DLBL CELL LINES TO THE B-2 INHIBITOR VENETOCLOX

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Background: The B-2 inhibitor venetoclax demonstrated significant single-agent activity in recent clinical trials of relapsed/refractory chronic lymphocytic leukemia (CLL). However, results in some other B-cell malignancies characterized by B-2 overexpression have not been equally impressive. This particularly refers to diffuse large B cell lymphoma (DLBCL), where only 18% of patients responded to treatment with venetoclax in a recent phase I clinical trial (Davids MS et al, J Clin Oncol. 2017).

Aims: Investigate whether the SYK inhibitor R406 can increase sensitivity of DLBCL in vitro to venetoclax.

Methods: The following cell lines were used: Ly1, Ly7, Ly18, DHL4, Toledo and BJAB (all GCB DLBCL) and U2932, DHLL2, Ly3, Ly10, HBL1 and TMDB (all ABC DLBCL). The percentage of apoptotic cells was determined by Annexin V/PI staining for flow cytometry analysis. Expression of BCL-2 family members was determined by immunoblotting or RQ-PCR analysis.

Results: In a recent study, we showed that MCL-1 increases the resistance of anti-IgM stimulated CLL cells to venetoclax, and that SYK inhibitors can effectively overcome this resistance by blocking B cell receptor (BCR)-mediated MCL-1 upregulation (Bojarczuk K et al. Blood. 2016). Since constitutive activation of the BCR pathway has been described in both ABC and GCB DLBCL (Davis RE et al, Nature 2010; Chen L et al, Cancer Cell. 2013), we investigated whether treatment with the SYK inhibitor R406 can sensitize DLBCL cells to venetoclax. Single-agent venetoclax had only modest activity against most DLBCL cell lines at concentrations ranging up to 0.25 μM (Figure 1). Substantial apoptosis induction (>20%) was observed in only 2 GCB (Ly1 and Ly18) and 2 ABC (U2932 and Ly10) cell lines. R406 as single agent had almost no effect on tumor cell viability, with only one cell line showing >20% apoptosis induction (HBL1). However, addition of R406 to venetoclax resulted in a dramatic increase in the percentage of apoptotic cells in six of the investigated cell lines (Ly18, DHL4, U2932, Ly10, HBL1 and TMDB). A synergistic effect was also observed with Ly1 using a lower concentration of venetoclax, whereas no effect or only an additive effect was observed in the remaining cell lines (Ly4, Ly7, Toledo, BJAB, RHLL2 and Ly3). Among these, only Toledo expressed similar levels of BCL-2 and venetoclax sensitive BCL-2 family proteins (Figure 2). A further 2 in the other cell lines were extremely low or undetectable. To understand the mechanisms how R406 increases the sensitivity of DLBCL cells to venetoclax, we evaluated changes in the expression of MCL-1 and other antiapoptotic BCL-2 family proteins that have been associated with venetoclax resistance. Five of the seven R406 + venetoclax sensitive cell lines (Ly1, DHL4, U2932, HBL1 and TMDB) showed a 20-45% reduction in MCL-1 levels following 24 hours culture with 2μM R406, whereas no changes were observed in Ly18 and Ly10. However, a substantial reduction in A1 levels was observed in Ly18 and U2932 cells, whereas no substantial changes in A1 and BCL-XL expression were detected in any of the other investigated cell lines. Finally, we also investigated the effects of R406 on expression of HRK, which is a propapoptotic BCL-2 family member that was recently shown to be induced by SYK inhibition in a subset of GCB DLBCLs (Chen L et al, Cancer Cell. 2013). A substantial increase in HRK expression (140-640%) was observed in 5 of the 7 R406 + venetoclax sensitive cell lines (Ly1, Ly18, DHLL4, U2932 and TMDB).

Figure 1.

E1376

VB EXPRESSION ASSESSMENT AND CLONALITY DETECTION IN T-CELL PROLYMPHOCYTIC LEUKEMIA (T-PLL) BY FLOW CYTOMETRY (FCM) AND NEXT GENERATION SEQUENCING (NGS): A COMPARISON OF BOTH METHODS

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Background: Vβ repertoire analysis can distinguish monoclonal from polyclonal (reactive) T-cell proliferations. The molecular quantification of clonal T-cell receptor (TR) gene rearrangements can also be used to record minimal residual disease (MRD) in T-cell malignancies. TR clonality can either be assessed by FCM employing Vβ antibody panels covering ~70% of the normal human TR repertoire or by molecular techniques like NGS with primers that amplify virtually all possible Vβ-Jβ rearrangements. T-PLL is the most common type of non-lymphocytic T-cell leukemia. Clonal TR gene rearrangements are detected in virtually all T-PLL by FCM or PCR from peripheral blood (PB) or bone marrow samples.

Aims: To compare the results of parallel TRB-based clonality analyses by FCM and NGS in T-PLL.

Methods: We investigated diagnostic PB leukocytes of 73 T-PLL patients with median lymphocytes at 66% (range 13-93; harboring T-cells at 97% (55-100)).

FCM of surface (not intracellular) Vβ expression was assessed by the IOTest Beta Mark kit (Beckman Coulter). Libraries for NGS were prepared using 100ng of DNA via a 2-step PCR and sequenced on the Illumina MiSeq (2x250bp, v2) with a median coverage of 17,908 reads (range 1,125–41,193)/sample. In the first PCR TRB rearrangements were amplified using TRB BIOMED-2 V- and J-region primers (van Dongen et al, Leukemia 2003). In the second PCR step, sequencing adaptors and sample-specific barcodes were added. Annotation of V, (D)- and J-regions of TRB sequences was done using ARResT/Interrogate (Bystří et al, Bioinformatics 2016).

Results: In all samples one or two dominant clonal TRB rearrangements were detected by NGS and represented in median by 83% of reads (range 15-90%). In 36/73 (49%) of these cases, also FCM demonstrated clonality. Interestingly, in 8/36 (22%) of cases the dominant Vβ by FCM differed from the molecular clonotype. In 5 of these cases the discrepancy was likely most accountable to a non-functional TRB clonotype detected by NGS corresponding to a bi-allelic TRB rearrangement with the second non-functional allele being preferentially identified by NGS. In 37/73 (51%) of cases no reaction with one of the Vβ antibodies was seen. In 16 (43%) of these cases this could be attributed to expression of a TRB rearrangement for which the appropriate Vβ antibody was not present in the FCM panel. In another 12 (33%) of these cases a non-productive TRB rearrangement represented the dominant NGS clonotype. However, in further 9 cases (24%), the functional TRB clonotype (TRBV 5-6, 6-5, 25-1, 18, 20-1, 27) was not detected by FCM despite theoretical coverage. Of note, overall 10/73 T-PLL (14%) lacked surface TRα/β chain expression.
IRF4 EXPRESSION IS ASSOCIATED WITH RESPONSE OF MANTLE CELL LYMPHOMA TO BRUTON’S TYROSINE KINASE INHIBITORS

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LIPIDS IN ADULT T-CELL LEUKEMIA/LYMPHOBLASTIC LEUKEMIA: IMPLICATIONS FOR TREATMENT RESPONSE.

Background: Mantle cell lymphoma (MCL) responds poorly to conventional chemotherapy. Inhibitors of Bruton’s tyrosine kinase (BTKi) have unexpectedly shown significant clinical effect; however despite this success, approximately one third of MCL patients have primary resistance to the drug, and patients who initially respond to treatment frequently acquire secondary resistance and aggressive relapse of the disease. Understanding how BTKi-resistance or sensitivity is mediated can identify new targets for therapy or predictive biomarkers of response. Using an in vitro model system we have identified the transcription factor IRF4 as a sensitive indicator for BTKi response in MCL cell lines and primary cells.

Aims: To identify molecules or pathways responsible for resistance to BTKi drug cytotoxicity in mantle cell lymphoma using cell line models and primary cells.

Methods: Primary cells and validated MCL cell lines (REC-1, G519, JEKO-1, JVM2) were cultured either alone, or together with murine stromal cells (with or without CD40L transfection). The BTKi sensitive REC-1 cell line was continuously treated with BTKi to generate an acquired resistance model. Cultures were treated with BTKi drugs: ibrutinib and acalabrutinib in the presence or absence of B-cell receptor or CD40L stimulation, and their sensitivity or resistance to treatment was determined using flow cytometry to assess proliferation (Ki67), apoptosis (Annexin-V), or phosphorylation of BTK (pY223). Changes affecting downstream proteins were determined by protein expression or phosphorylation analysis (immunoblotting) and by mRNA expression (RT PCR). Following initial experiments the studies focussed on IRF4.

Results: Each MCL cell line showed basal phosphorylation of BTK (Y223) and its downstream effector molecule ERK1/2 (Y204/187); in each case phosphorylation was prevented by BTKi. Of the cell lines tested however, only REC-1 cells showed growth inhibition by BTKi (ibrutinib and acalabrutinib), demonstrating both dose-dependent apoptosis (p<0.01) and inhibition of proliferation. Further investigation showed that only the BTKi-sensitive REC-1 cell line downregulated IRF4 in response to BTKi; this downregulation was an early and specific response (mRNA downregulated after 4 hours, and protein expression after 8 hours). Furthermore in REC-1 cells with acquired partial resistance to BTKi, the downregulation of IRF4 was significantly less than in the parental cell line. Finally in vitro culture of REC-1 cells with CD40L prevented IRF4 downregulation. Changes affecting downstream MCL cells reinforced these findings: in vitro CD40L induced proliferation, survival, prevented BTKi-induced IRF4 downregulation and protected the cells from BTKi-induced death. These findings were confirmed using ex vivo samples from treated patients (n=7) analysed before and during BTKi treatment. In some samples from patients shown to be clinically responding to BTKi and not downregulated in 1 refractory case. Summary/Conclusions: CD40L encountered in the cellular microenvironment supports the proliferation and survival of MCL cells, and protects them from the effects of BTKi inhibition. This study has identified that BTKi induces downregulation of IRF4 in sensitive but not resistant MCL cells, and that downregulation is opposed by CD40L. This suggests that the expression of IRF4 following treatment with BTKi might be a biomarker for BTKi-sensitivity in MCL, and that proteins modulated by IRF4 may play an important role in MCL treatment response.

E1379

LIQUID BIOPSY: DECIPHERING A SIGNATURE OF CIRCULATING MICRONAS AS NOVEL NON-INVASIVE BIOMARKERS IN DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Up to 40% of Diffuse Large B-Cell Lymphoma (DLBCL) patients still experience treatment failure or disease relapse after conventional chemotherapy. Therefore, the search of novel non invasive biomarkers able to early identify these patients is warranted in order to offer a different therapeutic approach. Recently, bodily fluids have emerged as an important source of information about its involvement in B lymphocyte biology and lymphomagenesis.

Aims: The aim of this study is to define the impact of and the mechanism by which TPL2 kinase affects MYC-induced lymphomagenesis.

Methods: CD19+ positive B lymphocytes were isolated from peripheral blood of healthy individuals and mouse B cells from spleens of WT (C57BL/6) and lymphoma transgenic mice engineered to overexpress c-myc in B cell progenitor cells under the control of the IgH chain enhancer. Mouse pre-B lymphocytes were isolated from bone marrow by flow cytometric cell sorting. Differential status of lymphomas was analysed by flow cytometry using B220, IgM and IgD antibodies. The TPL2 RNA and protein expression levels were assessed by qPCR and Western blot analysis, respectively. The extent of apoptosis was estimated by immunohistochemical evaluation of activated caspase-3 in paraffin embedded mouse lymphoma tissues and by flow cytometry using Annexin and 7AAD staining of ex vivo cultured lymphoma cells following cytobiotic deprivation.

Results: TPL2 RNA levels were found dramatically decreased in various human Burkitt lymphoma cell lines as well as in 7 primary Burkitt lymphoma biopsies compared to B lymphocytes of healthy individuals. In line with this finding, both pre-B and B lymphomas derived from Eμ-myc mice express very low levels of TPL2 mRNA and protein level, compared to pre-B and splenic B lymphocytes isolated from WT mice. Interestingly, pre-B and B lymphocytes of healthy (premalignant) Eμ-myc mice express TPL2 in comparable levels to their WT counterparts, suggesting that the reduction of TPL2 expression in lymphomas is an additional oncogenic alteration. In this regard, genetic ablation of TPL2 in Eμ-myc mice (Eμ-myc/tpl2−/−) significantly shortened their survival to 92 days from 140 days of Eμ-myc/tpl2+/+ mice (p<0.005). Eμ-myc/tpl2−/− mice also displayed a trend to develop more pre-B cell lymphomas compared to Eμ-myc/tpl2+/+ mice. This may be attributed to the decreased TPL2 expression in mouse pre-B lymphocytes, while it is upregulated in mature B lymphocytes. Finally, Eμ-myc/tpl2−/− lymphomas displayed reduced levels of apoptosis.

Summary/Conclusions: This study reveals a novel pathway during myc-driven lymphomagenesis. We show that MYC deregulation imposes selective pressure in favor of clones with decreased expression of TPL2 kinase. This process seems to be advantageous for the malignant clone, since genetic ablation of TPL2 in the Eμ-myc mouse model accelerates MYC-induced lymphomagenesis likely by contributing to apoptosis resistance.
Antibodies could be enhanced by manipulating metabolism. We identified and validated a serum miRNA signature with prognostic value in a cohort of newly diagnosed DLBCL patients. Methods: This is a go-on prospective non-interventionist study on a cohort of newly diagnosed de novo DLBCL patients uniformly treated with six courses of R-CHOP (Rituximab, Cyclophosphamide, Vincristine, Doxorubicin and Prednisone). Serum samples of patients were collected at diagnosis and after the end of treatment. Response treatment was evaluated by standard Cheson criteria. The expression profile of selected circulating miRNAs described as associated with lymphoid malignancies by us (let-7c/miR-99a/miR-125b cluster) and by previously published studies (miR-22, miR-18a and miR-20a) was evaluated by previously published microarray studies. Circulating-chromosomal samples collected at diagnosis of the first 18 patients enrolled into the study. Results: Our results showed that the expression level of serum miR-22 as well as let-7c/miR-99a/miR-125b cluster was significantly higher at diagnosis, in patients unresponsive to R-CHOP treatment when compared with responsive patients. On the contrary, miR-18 and miR-20 levels appeared to be not significantly associated to treatment response. In addition, a global expression profile of circulating miRNAs was evaluated in serum samples derived from a smaller cohort of patients (n=4) after first-line chemo-immunotherapy. Interestingly, we found a striking difference in miRNA modulation upon treatment between unresponsive and responsive patients. In particular, we found 31 miRNAs significantly modulated after R-CHOP in the group of responsive patients, including miR-22. In contrast, this miRNA subset did not show remarkable expression changes in unresponsive patients. Moreover, we performed a study interrogating The Cancer Genome Atlas (TCGA) database about miRNA expression levels in samples of DLBCL patients. We found that the only available data are relative to the miRNA expression levels in tumor tissue samples of 47 out of 58 DLBCL patients. Kaplan Meier method and log-rank test revealed a signature of 13 miRNAs with potential prognostic value. Among these we found that miR-22, also emerged as modulated in our genome-wide analysis, was linked to risk of disease recurrence. Summary/Conclusions: These preliminary data suggest that the serum miR-22 as well as miR-99a/let-7c/miR-125b miRNA cluster are of potential interest as non–invasive biomarkers to predict therapeutic response in DLBCL patients. Ongoing experiments in a wider cohort of patients are aimed to confirm these results and unveil potential miRNA signature with predictive value.

E1380
INTRACELLULAR CALCIUM AND METABOLISM HAVE CRITICAL ROLES IN DETERMINING ANTI-CD20 ANTIBODY EFFICACY IN DLBCL
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Background: Since the discovery and utilisation of the Type-I anti-CD20 antibody Rituximab, many have tried to enhance the efficacy of anti-CD20 antibodies in order to improve first-line treatment of B cell malignancies, leading to the development of Type-II anti-CD20 antibodies. To date, it is not clear what the biological role of CD20 and the mechanism of anti-CD20 antibody action remains unclear. However, CD20 has been shown to be involved in the store operated calcium (Ca2+) system. This complex has the ability to facilitate mitochondrial permeabilisation, resulting in reduced mitochondrial function. Basal oxidative phosphorylation (OxPhos), ATP production, and maximal and spare respiratory capacity of cells can be calculated as a measure of mitochondrial function. Aims: i) Assess and compare intracellular calcium concentration following treatment with anti-CD20 antibodies ii) Evaluate mitochondrial function of cells following treatment with anti-CD20 antibodies iii) Assess whether cytotoxicity of Type-I and Type-II anti-CD20 mAbs can be enhanced by exploiting cellular metabolism

Methods: We established a panel of four DLBCL cell lines (Karpas422, Pfeiffer, OCI-LY7 and SUDDL4). Following a 24-hour treatment with one of four anti-CD20 antibodies (Rituximab) and three Type-II anti-CD20 antibodies (BH2H, Obinutuzumab and Tositumomab), intracellular calcium concentration was quantified and visualised using imaging flow cytometry. Next, we used the XF Seahorse Mito Stress Test to reveal bioenergetic profiles of the cell lines following a 24-hour treatment with the same antibodies. We used Metformin to inhibit oxidative phosphorylation (OxPhos) and then characterised the bioenergetic profile of our panel of cell lines again, this time to assess how combining each anti-CD20 antibody with an OxPhos inhibitor affected mitochondrial function. Metformin was also used to reduce the mitochondrial membrane potential (MMP) across our panel of cell lines. We confirmed MMP reduction by staining cells with JC-1, a chameleon dye used as an indicator of MMP and analysed samples using flow cytometry. Under the same conditions, we conducted clonogenic survival assays to see whether cytotoxicity of anti-CD20 antibodies could be enhanced by manipulating metabolism.

Results: Intracellular calcium concentration was decreased across our panel of cell lines following a 24-hour treatment with all Type-II anti-CD20 antibodies in our panel. This decrease was not observed following treatment with the Type-I anti-CD20 antibody Rituximab. Treatment with anti-CD20 antibodies resulted in a significant increase in the maximal respiratory capacity of our panel of cell lines; cells were able to produce more ATP in response to oxidative stress. Conversely, we show that metabolic inhibition of OxPhos impaired mitochondrial function, causing a significant reduction in basal OxPhos and in maximal respiratory capacity. Under this condition, cells were unable to increase ATP production in response to oxidative stress. We also show that treatment combining Metformin with either Type-I or Type-II anti-CD20 antibodies prevents the increase in maximal respiratory capacity observed with anti-CD20 antibody treatment alone. When analysing the clonogenic survival of cell lines, we have found that only the cytotoxicity of Type-II anti-CD20 antibodies is enhanced by simultaneously treating cell lines with Metformin.

Summary/Conclusions: Our data show for the first time that when cells are unresponsive to Type-I anti-CD20 antibodies, intracellular calcium is decreased. Intracellular calcium remains unchanged following treatment with Rituximab. Next, we show anti-CD20 antibody treatment causes cells to increase maximal mitochondrial respiratory capacity to compensate for reduced basal mitochondrial function. We show that inhibition of OxPhos disables the cells from being responsive to oxidative stress. We also show that combining Metformin with Type-II anti-CD20 antibodies leads to enhanced cytotoxicity, with a significant reduction in clonogenicity in our panel of DLBCL cell lines.

E1381
CYCLIN D1 ONCOGENIC OVEREXPRESSION LEADS TO A GLOBAL TRANSCRIPTIONAL DOWNREGULATION IN MALIGNANT LYMPHOID CELLS
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Background: Cyclin D1 is an onco gene frequently overexpressed in human cancers. In hematologic neoplasms, mantle cell lymphoma and multiple myeloma are clear examples of deregulated cyclin D1 expression. It plays a dual function as cell cycle and transcriptional regulator, although the latter is widely unexplored.
Aims: In this study, we investigate the transcriptional role of cyclin D1 in lymphoma and its overexpression in B cells as a model of the first steps in MCL oncogenesis.
Methods: Chromatin immunoprecipitation (ChIP) followed sequencing was performed in four established MCL cell lines. RNA-sequencing (RNA-Seq) and information from histone ChIP-Seq were correlated with genomic intervals displaying cyclin D1 binding. Transcriptional downregulation was studied through cytotmetric RNA total quantification in lymphoblastic cyclin D1 overexpressing models and RNA Pol II ChIP-Seq.
Results: Endogenous cyclin D1 showed widespread binding to active promot- ers and was associated with a transcriptional down-regulation in several chromosomes. Indeed, cyclin D1, instead of showing specific gene activation, seems to globally decrease cell transcription. Mantle cell lymphoma and multiple myeloma cell lines displayed an inverse relation with cyclin D1 quantity. This transcriptional effect was associated with an increased RNA polymerase II pausing in promoters due to cyclin D1-overexpression over all transcriptional states.
Summary/Conclusions: This mechanism expands the oncogenic cyclin D1 functions and places the transcriptional machinery as a potential therapeutic target in cyclin D1 overexpressing tumors.

E1382
MICROENVIRONMENTAL EXPRESSION OF IMMUNOREGULATORY MOLECULES AND CYTOKINES IN CLASSICAL HODGKIN LYMPHOMA PROGNOSIS
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Background: Over the past decade, new biologic insights have revealed a key role of tumor microenvironment in the pathogenesis of classical Hodgkin's lymphoma (cHL). cHL infiltrating cells produce cytokines and growth factors that provide essential stimulatory signals for survival and proliferation of Hodgkin’s and Reed–Sternberg cells. Moreover, clinical behavior of cHL may be directly regulated by the cross-talk between tumor cells and infiltrating immune cells.

 haematologica | 2017; 102(s2) | 567
Aims: The aim of our study was to estimate the role of microenvironment expression of immunoregulatory molecules (PD-1 ligands, IDO) and cytokines (TGFB, IL-13) in clinical outcome of cHL. Methods: 74 patients (median age: 44, range: 17-71 years; males: 22, females: 52) were included in the study. 55.4% of patients were diagnosed with an early stage of HL, while 44.6% - with advanced stages. ABVD or BEACOPP (14/esc) were administered as a 1st-line therapy, while 78.3% of patients achieved CR (CR/PR), while 8.1% had progression of disease during the therapy. We recorded 14.8% relapses in patients after the 1st line therapy during the follow-up period (median duration – 36 months; range 6-66 months). PD-L1, PD-L2, IDO, TGFB, IL-13 mRNA expression levels were analyzed in fresh pre-treatment lymph node biopsies using qRT-PCR.

Results: Expression of PD-L2 ligands was heterogeneous across the samples and did not depend on histological variant or stage of cHL. Only 12.1% of patients (9/74) were PD-L1 negative and all but one of those cases had a CR and a long-term remission. Patients with PD-L1 overexpression tended to have a higher risk of relapse, comparing to low or negative PD-L1 expression (p=0.1). We did not find any significant association between PD-L2 expression level and clinical outcome of cHL. Expression levels of IDO, TGFB, IL-13 were evaluated in 39 cHL samples. 18.4% (7/38) patient were IDO positive and 81.6% (31/38) - IDO negative. The presence of IDO expression was associated with a higher risk of relapse in cHL patients (p=0.006). 85.7% (6/7) and 23.3% (7/30) of relapses were observed during the follow-up period in IDO+ and IDO-patients, respectively (p<0.05). The patients with double negative expression of PD-L1 and IDO were noted to have a favourable outcome of cHL. A 5-year event-free survival (EFS) ratio was 80% for double negative PD-L1+/IDO-patients vs 20% for double positive PD-L1+/IDO+ patients (p=0.008). IL-13 was expressed at various levels depending on the stage of cHL with the highest expression levels in advanced stages. A trend for a higher risk of relapse was observed for HL patients with increasing level of IL-13, (p=0.23). TGFβ-expression was correlated with histological variants of cHL, however multivariate analysis confirmed that TGFβ+ expression is a significant increase EFS in cHL patients with HRs of 6.7 [95% (CI) 1.3-2.1, p=0.04].

Summary/Conclusions: Our results suggest that tumor microenvironment plays an important role in clinical behavior of cHL. Hence, better understanding of molecular mechanisms of interaction between tumor and immune cells probably can provide us with a novel promising strategy for relapsed/refractory cHL treatment.

E1383

AN IN VIVO TRACEABLE AND MULTIPLEXING CRISPR/CAS9 GENOME EDITING SYSTEM

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Background: Gene gain of function and loss of function mutations, oncogene overexpression, gene amplification, chromosome deletion and epigenetic changes, may lead to lymphoma onset. The CRISPR-Cas9 genome editing system has become a feasible tool for exploring the functions of specific genes in different contexts. We want to use this technique to screen for lymphoma suppressor genes.

Aims: Construct an in vivo traceable and multiplexing CRISPR-Cas9 gene editing system, which is high efficient for studying in vivo functions of both individual genes or any given chromosome fragment.

Methods: Two retroviral vectors were constructed via molecular clone, one of which contains a locus for tandem U6-sgRNAs and inducible GFP reporter gene and the other contains Cas9 and IRTG. This system’s function of traceable and simultaneously mutate multiple gene efficiencies were validated in vitro. Eμ-myc HSPCs retrovirally transduced with sgp53 and Cas9 were transplanted into sublethally irradiated C57/BL6 mouse.

Results: Co-transduced cells can be tracked by the expression of GFP protein and multiple sgRNA can be efficiently introduced to the GFP-labeled cells for simultaneously mutating multiple genes or deleting a large chromosome fragment. Further we applied this system for both in vitro and in vivo genome editing. As an example, we show that Tp53 mutation accelerated Eμ-Myc driven lymphoma onset in vivo.

Summary/Conclusions: This traceable and multiplexing CRISPR/Cas9 system might be useful for various genome editing applications.
Aims: We studied the expression of NKp46 on a representative panel of GI T-CL to assess its diagnosis and prognosis value.

Methods: Using formalin-fixed paraffin-embedded tissue biopsies, we assessed NKp46 expression by immunohistochemistry (IHC) and investigated its clinical and biologic significance on 177 intestinal, 11 lymph node and 7 other biopsies from 84 CD or RCD patients (RCDI, n=20; RCDII, n=40), 44 GI T-cell lymphoma patients (EATL, n=25; monomorphic epitheliotropic intestinal T-cell lymphoma_MEITL, n=4; indolent T-LPD, n=15), 11 healthy patients and 5 patients with a GI inflammatory environment as controls.

Results: By doing ROC analysis on number of cells expressing NKp46 on GI-TCL we identify that 25 intra-epithelial lymphocyte (IEL) per 100 epithelial cells (EC) clearly separates RCDII from CD and RCDI patients, with a good positive and negative predictive values (100% and 95% respectively). In healthy controls, CD or RCDI patients, NKp46 was only expressed on scattered IEL (median 3%, 0-15). Based on NKp46 expression the overall survival is poor if over 25% of IEL are positive for NKp46 (OS-5years 96.4% vs 72.8%, P=0.0004) (Figure 1A). Among patients with GI T-cell lymphoma, we show that NKp46 was expressed in most of aggressive lymphoma (EATL 80%, n=20/25 and MEITL 100%, n=4/4). On the other hand, NKp46 was not expressed in most of indolent T-LPD (n=0/15). The NKp46 expression was also associated with a poor prognosis in GI T-cell lymphoma patients (OS-5years 50.5% vs 72.8%, P=0.0011) (Figure 1B).

Summary/Conclusions: The NKp46 expression in more than 25 IEL per 100 EC by IHC analysis can easily identify RCDII from CD and RCDI. Furthermore, the NKp46 expression is associated with aggressive forms of GI T-cell lymphoma. Finally, the NKp46 expression was strongly associated with shortened survival. Thus NKp46 provides a new biomarker for both diagnosis and prognosis in GI T-CL.
hours and produced 8.7 ~ 9.3 X 10^3 ng/ml of IgM). PCs isolated from BCWM-1 increased to 130% and produced 2.5 ~ 2.8 X 10^3 ng/ml of IgM. LPLs from both cell lines proliferated in culture (~130 ~ 140% in MWCL-1 and ~170 ~ 200% in BCWM-1 at 72 hours), gave rise to the more differentiated PCs (7.5 ~ 9.0% PCs at 72 hours in MWCL-1 and 1.2 ~ 1.4% PCs in BCWM-1), and secreted smaller amounts of IgM than PCs (3.5 ~ 5.0 X 10^3 ng/ml in MWCL-1 and 0.3 ~ 0.7 X 10^3 ng/ml in BCWM-1).

Summary/Conclusions: Our analysis of the 2 WM cell lines provides evidence to support the common hypothesis that malignant PCs arise from the clonal malignant LPL population, and are primarily responsible for IgM secretion in WM.

E1389
LMP-1 MEDIATED UPREGULATION OF IL-2RA PROMOTES LYMPHOMA-GENESIS AND CHEMOTHERAPY RESISTANCE IN NATURAL KILLER/T-CELL LYMPHOMA AND COULD BE A POTENTIAL THERAPY TARGET
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Background: Natural killer/T-cell lymphoma (NKTCL) is an Epstein–Barr virus (EBV)-associated, highly aggressive lymphoma. Treatment outcome remains sub-optimal, especially for advanced-stage or relapsed diseases. Our previous study demonstrated the prognostic value of IL-2Ra in NKTCL, but the role of IL-2Ra in the lymphomagenesis and chemotherapy resistance and its interactions with EBV in NKTL should remain to be investigated.

Aims: This study investigated the mechanism of IL-2Ra expression in NKTCL, and explored the role of IL-2Ra in lymphomagenesis and chemotherapy resistance as well as the potential role of anti-IL-2Ra treatment in NKTL.

Methods: Expression of IL-2Ra was measured in NK-92 (LMP-1 weak expression) and SNK-6 (LMP-1 strong expression) cells by western blot, quantitative real-time PCR, enzyme-linked immunosorbent assay, and flow cytometry, respectively. LMP1-harboring lentiviral vectors were transfected into NK-92 cells to examine the correlation between LMP1 and IL-2Ra expression. Proteins in the downstream pathway of LMP1 signaling were measured in NK-92 cells transfected with LMP1-harboring or negative control vectors as well as in SNK-6 cells. Then IL-2Ra-harboring lentiviral vectors were transfected into both NK-92 cells and SNK-6 cells to examine the cell proliferation by CCK8, apoptosis by staining with Annexin V and detected by flow cytometry (FCM), cell cycle distribution by FCM analysis, and IC50 values exposed to three chemotherapy drugs (adriamycin, gemcitabine, and asparaginase) by MTT. Finally anti-IL-2Ra antibody was added to investigate its ability of reversal of drug resistance.

Figure 1.

Results: Expression of IL-2Ra was significantly upregulated in SNK-6 cells than in NK-92 cells, at both protein and mRNA levels. Expression of IL-2Ra was remarkably upregulated in NK-92 cells transfected with LMP1-harboring lentiviral vectors compared with those transfected with negative control vectors. Proteins in the MAPK/NF-κB pathway were upregulated in LMP1-expressing NK-92 cells compared with the negative control. Selective inhibitors of those proteins induced the MAPK/NF-κB pathway were upregulated in LMP1-expressing NK-92 cells and as in SNK-6 cells. When comparing with those transfected with negative control vectors, cell growth was significantly increased in both NK-92 and SNK-6 cells transfected with IL-2Ra-harboring lentiviral vectors, and the cell cycle assay displayed a significant decrease in the percentage of cells in the G1/S phase (p<0.05) and an increase in the percentage of cells in the S phase (p<0.05), while apoptosis was not affected. Subsequent western blot tests demonstrated that cyclin A, B, D, and CDK1, 4 were involved in the regulation of cell cycle with overexpression of IL-2Ra. The IC50 values to all three chemotherapy drugs were significantly increased after overexpression of IL-2Ra (p<0.05), which can be fully reversed by anti-anti-IL-2Ra antibody.

Summary/Conclusions: IL-2Ra expression was upregulated in NKTCL by LMP-1-mediated activation of MAPK/NF-κB pathway. IL-2Ra can promote NKTCL cell proliferation partially through regulation of cell cycles and induce chemotherapy resistance, which can be reversed by anti-IL-2Ra antibody, indicating the potential role of IL-2Ra as a therapy target in NKTCL.

E1390
LENALIDOMIDE (LEN) DRIVES PROGRAMMED DEATH-1 (PD1) PATHWAY UPREGULATION IN A TUMOR MICROENVIRONMENT (TME) MODEL OF ACTIVATED LOW-GRAD E LYMPHOMA CELLS
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Background: Hodgkin lymphoma (HL) is a B-cell non-HLTLT-cell malignancy that predominantly affects young adults. PD1/PD-L1 axis has been extensively investigated on lymphoma cells, however, the PD1/PD-L1 axis in the microenvironment of HL remains less understood.

Aims: 1) To better characterize the PD1/PD-L1 and the lesser-known PD-L2, phenotype in peripheral neoplastic CD19+ lymphocytes and T-cell subsets in patients with low-grade B-cell lymphoma. PD1/PD-L1/PD-L2 expression on HL was not completely explored. 2) To determine whether PD1/PD-L1/PD-L2 cytotoxicity in experimental models Preclinical findings indicate that combination of IMIDs with immune checkpoints inhibitors may promote therapeutic synergy and long-term antitumor immunity to improve clinical outcome.

Methods: Samples obtained from patients attending participating Hematology Units were used to determine PD1, PD1L, PD2L phenotype (%SEM) by Flow-cytometry (FC). Autologous activated cells (AAT) were obtained by co-culture of patient T-cells with anti-CD3/CD28 beads, rIL2 and with PBMCs. Cultures were monitored daily until sizeable clumping was observed and tested for PD1 and ligand expression. In selected experiments LEN(presented using Cell-ex) was added to cell cultures.

Results: Twelve cases of lymphoma were evaluated for PD1, PD1L and PDL2 expression on malignant B- and T-cells by FC. The expression of PD1 and PDL2 was similarly expressed, while PD1L was almost undetectable on B-cells. Levels of PD1 expression on CD3+ cells were variable across samples, however they were significantly higher than those expressed on malignant B-cells. Significantly higher PD1 expression and very low levels of ligands were detected in both CD4+ and CD8+ cells. Co-expression of PD1, PD-L2 and PD-L1 in consistent formation of B/T-cell clusters. Higher numbers of CD19+CD29+PDL2+ cells were detected than PDL1+ cells compared to baseline cells. PD1 expression also significantly increased in AAT co-culture on B-cells. PD1 expression...
on CD3+ cells was unaffected by AAT, although the expression of both ligands increased significantly. Closer analysis of T-cell subsets showed that only in CD4+ cells, PD1 expression increased significantly following co-culture experiments. Preliminary data on lymphoma-AAT co-culture experiments (n=3) indicated that LEN (0.5/1 μM) did not negatively influence the formation of AAT clusters. After 48 h of co-culture, the expression of CD19+CD5-CD11c+ cells increased in 2/3 cases following LEN treatment while, PDL2 expression remained unchanged. PD1 expression gradually increased following exposure to LEN compared to untreated cells. CD3+ cells showed a significant increase in PD1 expression by LEN, while the expression of both ligands remained unaffected. Evaluation of activated T-cell subsets showed similar results, with the exception of stronger induction of PD1 and PDL1 expression by LEN in CD8+ cells.

Summary/Conclusions: Our data provide support for the potential involvement of the PD1-axis in lymphoma patients. Interestingly, LEN further induces the expression of PD1 in CD8+ and CD4+ cells and may contribute to reactivating PD1 signaling under treatment. The PD1 pathway may be potentially targeted to overcome both the intrinsic and LEN-induced exhaustion phenotype.

E1391
IDENTIFICATION AND DIAGNOSTIC APPLICATION OF GENOMIC NPM-ALK FUSION SEQUENCES IN ANAPLASTIC LARGE CELL LYMPHOMAS
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Background: ALK positive anaplastic large-cell lymphomas (ALCL) account for 10-15% of pediatric Non-Hodgkin lymphomas. Most of these patients carry the chromosomal translocation t(2;5)(p23;q35) resulting in the gene formation. The quantification of NPM-ALK fusion transcripts is a well-established tool for diagnostic purposes and risk stratification during the course of treatment.

Aims: Establishment of a PCR based assay to identify patient-specific genomic NPM-ALK fusion sequences for a DNA based monitoring of minimal residual disease in ALCL patients. Compared to RNA based methods the quantification of DNA is independent of the gene expression. Additionally, due to the higher stability of DNA, cell-free circulating tumor DNA (ctDNA) should be detectable in the patient’s plasma and may represent a tumor marker for “liquid biopsies” in ALCL.

Methods: Using a specifically designed multiplex long-range PCR assay, genomic NPM-ALK fusion sequences were identified in 45 ALCL patients. The genomic NPM-ALK breakpoints were analyzed concerning fine structure and breakpoint distribution pattern. Furthermore, the patient-specific genomic NPM-ALK fusion sequences were evaluated for their use as biomarkers in selected cases. For this purpose patient’s blood and plasma samples were quantified using a high sensitive digital droplet PCR assay.

Results: In more than 60% of cases the identified breakpoint was localized within repeat regions. The genomic breakpoints within the breakpoint cluster regions of the fusion genes were randomly distributed. Most of the NPM-ALK fusion sequences were characterized by the occurrence of small insertions or deletions indicating the involvement of the non-homologous end-joining (NHEJ) repair system for chromosomal translocation initiation. Using a DNA based quantification assay in a subset of patients, the genomic NPM-ALK fusion sequences were detectable in circulating tumor cells in patient’s blood samples as well as cell-free tumor DNA in plasma samples.

Summary/Conclusions: The established multiplex long-range PCR assay is a useful diagnostic tool for the identification of genomic NPM-ALK fusion sequences. This individual tumor maker is independent of gene expression and can be used for therapy response monitoring and relapse detection.

E1392
ARSENIC TRIOXIDE TARGETS BCL6 FOR DEGRADATION AND INHIBITS THE PROLIFERATION OF BCL6-DEPENDENT DIFFUSE LARGE B-CELL LYMPHOMA
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Background: B-cell lymphoma 6 (BCL6) is a transcription repressor and is frequently over-expressed in diffuse large B-cell lymphoma (DLBCL). It suppresses the expression of its target genes ATR, TP53 and CDKN1A, leading to dysregulation of DNA repair and cell proliferation. It has been shown that BCL6 is an oncoprotein involved in the pathogenesis of DLBCL and represents a potential therapeutic target. Arsenic trioxide (ATO) targets various oncogenic proteins, including PML-RARA in acute promyelocytic leukemia (APL). Tax in adult T-lymphoblastic leukemia (ATL), cyclin D1 in mantle cell lymphoma (MCL), and NPM-ALK in anaplastic large cell lymphoma (ALCL), for degradation through the ubiquitin-proteasome pathway. ATO is now used for the management of ATL and MCL with proven clinical benefit.

Aims: To investigate if ATO targets BCL6 and inhibits the proliferation and growth of BCL6-dependent DLBCL.

Methods: BCL6-dependency of a panel of DLBCL cell lines (i.e. OCI-Ly1, OCI-Ly7, SU-DHL-6, OCI-Ly18 and Pfeiffer) was determined based on their sensitivity to proliferation inhibitory activity of the BCL6 inhibitor 79-6 (Calbiochem). The effects of ATO and cisplatin as single agent or in combination on cell viability and apoptosis of DLBCL cells were examined with trypan blue dye exclusion assay and flow cytometric analysis. Expression of BCL6 and its target genes was examined with quantitative RT-PCR and western immunoblotting. The therapeutical efficacy of ATO treatment was also examined in a DLBCL (OCI-Ly7) xenograft mouse model.

Results: OCI-Ly1, OCI-Ly7 and SU-DHL-6 were highly sensitive to inhibitory activity of BCL6 inhibitor and were designated as BCL6-dependent. Treatment of DLBCL cells with ATO led to a decrease in BCL6 protein level and an upregulation of downstream targets of BCL6, including PRDM1, CD44 and CD69. The effect of ATO on BCL6 protein were abrogated by treatment with proteasome inhibitor MG132. Similar results were observed in ATO treated BCL6 for degradation through the ubiquitin-proteasome pathway. Interestingly, ATO also inhibited cell proliferation and induced apoptotic cell death of BCL6-dependent DLBCL cell lines, analogous to the effect of BCL6 inhibitor on these cells. In addition, there was a synergistic inhibitory and cytotoxic activity between ATO and cisplatin. Finally, ATO treatment suppressed the growth of DLBCL in a xenograft mouse model.

Summary/Conclusions: ATO targets BCL6 for proteasomal degradation and inhibits the proliferation and growth of BCL6-dependent DLBCL.

E1393
PROTEOMIC PHOSPHOSITE ANALYSIS IDENTIFIED CRUCIAL NIPA SERINE RESIDUES FOR NPM-ALK-MEDIATED TRANSFORMATION
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Background: Anaplastic large-cell lymphoma (ALCL) is an aggressive non-Hodgkin lymphoma that occurs mainly in children and younger adults. Patients typically show an advanced stage disease as well as an aggressive disease pattern with typical lymphomatous manifestations. At the molecular-genetic level, 60% of the patients with systemic ALCL exhibit a translocation t(2;5)(p23;q35), which leads to the expression of the NPM-ALK fusion protein. Under the control of the NPM promoter, ALK activation causes increased and autonomous cell proliferation. Nuclear interaction partner of ALK (NIPA) was first identified as a new interaction partner of the oncogene NPM-ALK in a yeast-2-hybrid screen which defines an E3-SCF ligase and is physiologically involved in cell cycle regulation at the transition from G2 phase to mitosis. It has already been shown in preliminary studies that co-expression of NIPA with the oncopgenic tyrosine kinase NPM-ALK results in the constitutive phosphorylation of NIPA (Iler et al., 2012a).

Until now, the specific signal transduction pathway, the crucial phosphorylation sites as well as the functional effect of the pathological NIPA phosphorylation in NPM-ALK-induced lymphomagenesis still remain unclear. Molecular insights into the activated pathways of the oncogenic tyrosine kinase NPM-ALK may help to identify new druggable targets for therapeutic implications.

Aims: In the present study, we investigated the molecular mechanisms as well as the functional impact of the NPM-ALK-induced NIPA phosphorylation.

Methods: For this purpose, biochemical methods with ALCL cells were used to examine functional effects of constitutive and NIPA phosphorylated NPM-ALK. Moreover, we performed a "proteomic-phosphosite-analysis" to identify crucial NPM-ALK specific phosphorylation sites in NIPA. Based on these results, phosphorylation-deficient NIPA mutants were generated to investigate the functional effect of this phosphorylation: MTJ proliferation- and Softagar-Assays were performed after retroviral infection of Ba/F3 and primary NIPA-deficient MEF cells with NPM-ALK and the respective phosphate-deficient NIPA to reveal transformation and growth ability.

Results: It has already been shown, that cell cycle dependent NIPA phosphorylation at critical serine residues 354, 359 and 395 leads to dissociation of the interaction. In vitro, we were able to show that the NIPA constitutive phosphorylation of NIPA by NPM-ALK does not lead to changes in the SCFPNP complex formation. Proteomic-Phosphosite-analyses identified 10 significantly upregulated (ratio >2; Log2Fold Change) NIPA phosphosites upon NPM-ALK-mediated phosphorylation. The phosphorylation-deficient NIPA mutants were generated to investigate the functional effect of this phosphorylation: MTJ proliferation- and Softagar-Assays were performed after retroviral infection of Ba/F3 and primary NIPA-deficient MEF cells with NPM-ALK and the respective phosphate-deficient NIPA to reveal transformation and growth ability.

Summary/Conclusions: Taken together, we identified five phosphorylation sites in NIPA to be highly upregulated upon NPM-ALK expression. However,
NPM-ALK mediated NIPA-phosphorylation of those sites did neither change the SCF/Stat3 signal in vitro nor influence the NIPA localization at the nuclear pore complex, but silencing of these NIPA Serine/Threonine residues led to significantly reduced proliferation and altered transformation ability of Ba/F3 and primary MEF cells. Further analyses will shed some light into the mechanisms underlying these findings and evaluate NIPA as a possible new treatment option for ALCCL.

E1394
APPLICATION OF CELL-OF-ORIGIN SUBTYPES DETERMINED BY DIGITAL GENE EXPRESSION IN HIV-RELATED DIFFUSE LARGE B CELL LYMPHOMAS

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Background: Diffuse large B cell lymphoma (DLBCL) can be divided according to cell of origin (COO) in germinal center B-cell-like (GCB) and activated B-cell-like (ABC) that have shown different prognostic. Immunohistochemistry (IHC)-based algorithms and recently, Lymph2Cx assay, a digital test based on cell of origin (COO) in germinal center B-cell–like (GCB) and activated B-cell (ABC).

Methods: A series of 55 patients with the diagnosis of HIV-related DLBCL infected patients has been scarcely studied. In DLBCL arising in immunocompetent individuals, its applicability on HIV-infected patients has been carefully studied.

Aims: To study the characteristics and prognostic impact of COO subtypes in a series of HIV-related DLBCL using the Lymph2Cx assay and to compare the results with those obtained with an IHC-based algorithm.

Results: The median follow-up of living patients was 8.5 years. IHC studies showed that 35.8% of the cases expressed CD10, 61.5% expressed BCL6, 55.8% expressed MUM1, and according to Hans algorithm 56.6% had a non-GC phenotype. CD30 was expressed in 15.4% of the cases and EBER was found in 21.2%. The expression of MYC was detected in 32.7% of the cases and BCL2 in 44%, and 18% were dual expressers. Rearrangements involving MYC, BCL2 and BCL6 were detected in 26%, 8% and 28%, respectively. The Lymph2Cx assay assigned a COO to all 55 studied cases, 63.6% were GCB subtype, 20% were ABC subtype, and 16.4% were unclassified. The only clinical feature significantly associated with a defined COO subtype was B-symptoms (ABC=81.8% vs GCB=28.6%, P=0.003) and HIV-load tended to be more frequently observed in ABC (90%) than in GCB (58.1%, P=0.068). Regarding IHC and FISH results, MYC rearrangements were only detected in GCB cases and expression of CD10 and BCL6 tended to be associated with GCB (Table 1). Hans algorithm and Lymph2Cx assay differently assigned COO subtypes (κ=0.288, P=0.029) showing that 44.1% of the GCB cases had a non-GC phenotype according to Hans. Only patients treated with RCHOP were considered in survival analyses (N=47). COO subtypes had neither impact on OS nor PFS, independently of being determined with Hans or Lymph2Cx assay. Features associated with shorter OS and PFS were history of AIDS-defining illnesses, HCV-infection and dual MYC and BCL2 expression. Extranodal disease and increased MYC or BCL2 expression were also bad prognostic factors for PFS.

Summary/Conclusions: In HIV-related lymphomas, COO subtypes were discordantly assigned with Hans and Lymph2Cx assay and COO subtypes showed no impact on outcomes, independently of the method applied.

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E1395
CXCR4 AND CXCL12 ARE IMPLICATED IN BONE MARROW INFILTRATION PROCESS OF AGGRESSIVE B CELL LYMPHOMAS AND THEIR INHIBITION SUPPRESSES LYMPHOMA CELL GROWTH IN VITRO

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Background: The chemokine receptor CXCR4 together with its prime ligand CXCL12 plays a pivotal role in tumorogenesis of solid and haematological neoplasms. Our comprehensive study on the CXCR4 expression in aggressive lymphoma demonstrated that high CXCR4 expression was associated with poor clinical course of aggressive lymphoma patients.

Aims: Therefore, we aimed to comprehensively study the implication of the CXCR4 - CXCL12 axis in bone marrow infiltration process of aggressive lymphoma and to analyse the effects of CXCR4 antagonists on cell growth and migration of aggressive lymphoma cells in vitro.

Methods: To determine whether CXCR4 and CXCL12 expression have any effects on the bone marrow infiltration process of aggressive lymphomas, we performed gene expression analysis on bone marrow biopsies of our diffuse large B-cell lymphoma patient cohort. Therefore, we used 63 bone marrow specimens, whereby 52 bone marrow biopsies were taken at time of diagnosis. Additionally, we generated a novel CXCR4 antagonist -named WK1- by modification of the side chain of AMD070 - a commercially available CXCR4 antagonist. We treated various aggressive lymphoma cell lines (U2932 and RI-1 as ABC-DLBCL and BL2 and Raji as Burkitt lymphoma lines) with CXCR4 antagonists (AMD070 and its derive WK1 and the FDA approved CXCR4 antagonist AMD3100) and determined cell growth by using the EZ4U assay. Transwell migration using the Boyden chamber was used to estimate migration effects on the bone marrow infiltration process of diffuse large B-cell lymphomas. Additionally, we performed in vitro experiments that CXCR4 and its ligand CXCL12 is implicated in the bone marrow infiltration process of diffuse large B-cell lymphomas. Moreover, our in vitro results indicate that treatment of lymphoma cells with CXCR4 antagonists might be a promising new therapeutic intervention to eliminate lymphoma cells.
EPSTEIN-BARR VIRUS LOAD IN PLASMA IS AN EARLY BIOMARKER OF HIV-RELATED LYMPHOMA

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Background: Epstein Barr virus (EBV) has been detected in the tumor cells of some non-Hodgkin lymphomas (NHL) and Hodgkin lymphomas (HL) and detectable EBV loads have been found in the plasma of immunocompetent patients with HL. In HIV-related lymphomas the importance of EBV load as potential lymphoma biomarkers has been scarcely studied.

Aims: We aimed to evaluate the usefulness of EBV load in plasma as lymphoma biomarker in HIV-infected patients.

Methods: One hundred and fifteen patients with NHL (HIV-infected = 57 and HIV-uninfected = 34) and HL (HIV-infected = 16 and HIV-uninfected = 8) were studied. EBV loads were determined in plasma by means of a commercial real-time PCR technique (EBV PCR kit, Qiagen GmbH, Hilden, Germany) at lymphoma diagnosis and in a group of HIV-infected patients also at one year before diagnosis (N=11) and at complete response (CR) (N=34). EBV expression was studied by in situ hybridization in tumor biopsies. The following clinical and biological parameters were collected from records: age, gender, date of lymphoma diagnosis, ECOG score, extranodal and bulky disease, B symptoms, Ann-Arbor stage, serum lactate dehydrogenase and beta2-microglobulin, International Prognostic Index (IPI), HCV and HBV serology, history of opportunistic infection and of AIDS-defining illness, onset of combination antiretroviral therapy, CD4 counts, HIV loads, type and date of response, relapse date, last follow up or death date. McNemar’s test and Wilcoxon test were used to compare quantitative and qualitative variables, respectively. Survival analyses were performed using the Kaplan-Meier method. P-values of less than 0.05 were considered statistically significant.

Results: At diagnosis, EBV loads were detectable in more HIV-infected patients than HIV-uninfected (48% vs 14%, P<0.002) and in more HL cases than NHL (70% vs 26.3%, P=0.006). In HIV-infected patients, detectable EBV load was associated with EBER expression, 66.6% of the patients with detectable EBV loads had EBER-positive tumors and 92% of the patients with undetectable EBV loads had EBER-negative tumors (P=0.003). All the remaining clinical and biological features were not associated with detectable EBV load in plasma. In HIV-uninfected patients, associations between EBV load and EBER expression (P=0.006) and EBV load and HIV infection (P=0.017) were observed. From 16 out of 34 (47%) HIV-infected patients with detectable EBV loads at lymphoma diagnosis, 15 had undetectable EBV loads at CR (P=0.001) (Figure 1). The exception was one patient with HL whose EBV load substantially decreased at CR but was still detectable. Moreover, 4 out of 7 HIV-infected patients with detectable EBV loads at diagnosis had detectable loads one year before diagnosis, and no patient with negative EBV loads at diagnosis had detectable loads before it, pointing EBV load can be used as an early biomarker of lymphoma. EBV loads at diagnosis had neither impact on overall survival nor progression-free survival.

Summary/Conclusions: EBV-load in plasma can be used as early biomarker of lymphoma in HIV-infected patients since EBV-loads can be detected up to 1 year before lymphoma diagnosis and are virtually undetectable at lymphoma CR.

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E1387

CLONOTYPE AND MUTATIONAL PATTERN IN TCRG D LARGE GRANULAR LYMPHOCYTE LEUKEMIA

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Background: T-cell large granular lymphocyte leukemia (T-LGLL) is a rare heterogeneous T-cell neoplasia whose leukemic cells usually express the β3 T-cell receptor (TCR); only a small subset of cases expresses the γδ TCR denoting the TCRγδ LGLL. Currently, among the different LGL diseases, TCRγδ LGLL remains less studied and several clinical and laboratory data already described in TCRβ-LGLL have not yet been explored in TCRγδ-LGLL.

Aims: The aims of this work were 1) to characterize TCRγδ-LGLL defining STAT mutational pattern and CDR3 repertoire diversity/clonal composition (clonotype) and 2) to evaluate correlations among LGL phenotype, mutations, TCR rearrangement and clinical presentations.

Methods: In this work 11 patients affected by TCRγδ-LGLL were included. Sanger sequencing was used for mutational analysis on hot-spot regions in the two genes more frequently mutated in LGL disorders, STAT3 and STAT5b. Immunophenotype of LGL clone was defined by flow cytometry analysis. CDR3 repertoire and frequency distribution of TCR gamma gene rearrangements were evaluated.

Results: Our results showed that TCRγδ LGLL had a high incidence of STAT mutations, 9 out of 11 patients carrying STAT3 or STAT5b mutations in a mutually exclusive pattern. At variance from CD8+ TCRαβ LGLL and CD4+ TCRδ LGLL, most patients were first being monomorphic characterized by STAT5b mutations in the latter by STAT5b, TCRγδ LGLL patients were characterized by both the mutations. Thus, TCRγδ LGLL showed features shared by CD8 and CD4 TCRαβ-LGLL. Consistently, TCRγδ LGLL showed the same correlation between immunophenotype and kind of mutation observed in TCRαβ-LGLL: γδLGL patients with CD68+/CD56- LGL immunophenotype were characterized by STAT3 mutations (as in CD8+ T-LGLL), while γδLGL patients with CD56+ LGL immunophenotype by STAT5b mutations (as in CD4+ T-LGLL). Moreover, we observed that patients with γδLGLs positive for Vδ2 showed usually indolent course, whereas Vδ1 was linked to a more symptomatic disease (4 out of 5 symptomatic patients were Vδ1+), whereas no correlation was found between mutations and clinical course. By NGS of TCR gamma gene, we observed that all patients were clonal but two, showing a polyclonal pattern borderline with clonality percentage defined by sequencing kit criteria. Interestingly, these two last patients were the only two patients without STAT mutations. As far as the remaining cases are concerned, among 11 TCRγδ-LGLL patients (n=4), 3 were polyclonal and one biclonal, while STAT5b mutated patients (n=5) were more frequently monoclonal (4/5 monoclonal and 1/5 biclonal). In terms of clonal rearrangements, Vδ3-Jγ3/2, Vγ9-Jγ3P and Vδ8-Jγ3/2 were the combination usages most frequently detected. Concerning the clonotype repertoire, CDR3 sequences of the most prominent clonotype present in low frequency in almost all the other γδ patients and two different CDR3 sequences were found shared, each one in two different patients at frequency >10% of the total rearrangements.

Summary/Conclusions: Our data indicate that TCRγδ LGLL can be considered as the intercalation of the two types of TCRαβ-LGLL, sharing CD4+ and CD8+ T-LGLL mutational features. As already described in TCRαβ-LGLL, also in γδLGL patients the only two patients without STAT mutations. As far as the remaining cases are concerned, among 11 TCRγδ-LGLL patients (n=4), 3 were polyclonal and one biclonal, while STAT5b mutated patients (n=5) were more frequently monoclonal (4/5 monoclonal and 1/5 biclonal). In terms of clonal rearrangements, Vδ3-Jγ3/2, Vγ9-Jγ3P and Vδ8-Jγ3/2 were the combination usages most frequently detected. Concerning the clonotype repertoire, CDR3 sequences of the most prominent clonotype present in low frequency in almost all the other γδ patients and two different CDR3 sequences were found shared, each one in two different patients at frequency >10% of the total rearrangements.

E1398

INCREASED EXPRESSION OF IRF8 IN TUMOR CELLS INHIBITS THE GENERATION OF TH17 CELLS AND PREDICTS UNFAVORABLE SURVIVAL OF DIFFUSE LARGE B CELL LYMPHOMA PATIENTS

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Background: The immunological pathogenesis of diffuse large B cell lymphoma (DLBCL) remains elusive. Searching for new prognostic markers of DLBCL is a crucial focal point for clinical scientists.

Aims: The aim of the present study was to examine the prognostic value of interferon regulatory factor 8 (IRF8) expression and its effect on the development of Th17 cells in the tumor microenvironment of DLBCL patients.

Methods: Flow cytometry, immunohistochemistry, and quantitative real-time PCR were used to detect the distribution of Th17 cells and related cytokines and IRF8 in tumor tissues from DLBCL patients. Two DLBCL cell lines (OCI-
LY10 and OCI-LY1) with IRF8 knockdown or overexpression and two human B lymphoblast cell lines were co-cultured with peripheral blood mononuclear cells (PBMCs) in vitro to determine the effect of IRF8 on the generation of Th17 cells. Quantitative real-time PCR and Western blotting were used to investigate the involvement of retinoic acid receptor-related orphan receptor gamma t (RORγt) in the effect of IRF8 on Th17 cell generation. The survival of 67 DLBCL patients was assessed using the Kaplan-Meier method and Cox proportional hazards regression.

**Results:** The percentage of Th17 cells was lower in DLBCL tumor tissues than in PBMCs and corresponding adjacent benign tissues. Relative expression of interleukin (IL)-17A was lower, whereas that of interferon (IFN)-γ was higher in tumor tissues than in benign tissues. Co-culture with DLBCL cell lines inhibited the generation of Th17 cells in vitro. IRF8 upregulation was detected in DLBCL tumor tissues, and it was associated with decreased DLBCL patient survival. Investigation of the underlying mechanism suggested that IRF8 upregulation inhibited Th17 cell generation by suppressing the effect of RORγt on CD4+ T cells.

**Summary/Conclusions:** Our findings suggest that IRF8 expression in the tumor microenvironment inhibited the generation of Th17 cells through its antagonistic effect on RORγt in the DLBCL tumor microenvironment, suggesting that it could be a prognostic factor for DLBCL.

**E1399**

**GENOMIC PROFILING OF BCL2 AND MYC DOUBLE EXPRESSOR DIFFUSE LARGE B CELL LYMPHOMA**

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**Background:** Diffuse large B-cell lymphoma (DLBCL) is an aggressive disease featuring heterogeneous genetic, phenotypic and clinical characteristics. Recently, a negative prognostic impact of double expression of BCL2 and MYC (double expressor (DE) lymphoma) has been identified in several studies. SNP array (SNP-A) studies have already led to the identification of novel genomic aberrations in ABC and GCB subtypes of DLBCL whereas similar analysis has not been done in DE and non-DE DLBCL.

**Aims:** To characterize the landscape of genomic aberrations in DE and non-DE DLBCL groups using SNP-A and interphase fluorescence in situ hybridization (FISH).

**Methods:** Immunohistochemical and FISH analysis was performed on tissue microarray of formalin fixed paraffin embedded (FFPE) tumor tissue samples using Bcl2 (124, DakoCytomation) and MYC (Y69, Epitomics) antibodies and FISH MYC (Zytovision), Bcl2 (Abbott/Vysis), Bcl6 (Abbott/Vysis) break-apart probes and MYC (Zytovision) double fusion probe. Infinium HD genome-genotyping assay with the HumanCytoSNP SNP-FISH 12 BeadChip (Illumina Inc., San Diego, CA, USA) was performed for genomic analysis of the aberrations.

**Results:** A cohort of 91 primary DLBCL patients diagnosed between 2004 and 2012 was selected for the study. Immunohistochemical evaluation was informative in 81 (90%) of the patients. Double expressor (DE) lymphoma was detected in total 164 (50%) and 2 (1%) cases, respectively. 3q gain was detected in 59 (66%) cases, 10q deletion and 3q gain. Complex karyotype (>3 aberrations) was detected in 20 (25%) cases. 11q deletions were more prevalent in DE than in non-DE (16 vs 6q and 17q deletions were more prevalent in the non-DE had group. 6q and 17q deletions were more prevalent in the non-DE had group.

**Aims:** To assess the clinical significance of genomic abnormalities for patients with DLBCL. The clinical significance of different genomic aberrations for response and survival in a cohort of 91 patients with DLBCL was assessed.

**Methods:** Genomic profiling was assessed using SNP-A analysis. The SNP-A analysis detected a total of 329 genetic abnormalities not corresponding to known copy number polymorphisms (89% of all the patients, 59/66). These comprised 164 (50%) hemizygous and 2 (1%) duplications. The study included chemonaive DLBCL pts (Ann Arbour I-IV stages).

**Results:** The SNP-A analysis detected a total of 329 genetic abnormalities not corresponding to known copy number polymorphisms (89% of all the patients, 59/66). These comprised 164 (50%) hemizygous and 2 (1%) duplications. The study included chemonaive DLBCL pts (Ann Arbour I-IV stages). The SNP-A analysis detected a total of 329 genetic abnormalities not corresponding to known copy number polymorphisms (89% of all the patients, 59/66). These comprised 164 (50%) hemizygous and 2 (1%) duplications. The study included chemonaive DLBCL pts (Ann Arbour I-IV stages). The SNP-A analysis detected a total of 329 genetic abnormalities not corresponding to known copy number polymorphisms (89% of all the patients, 59/66). These comprised 164 (50%) hemizygous and 2 (1%) duplications.
vival (OS). The efficacy of R-CHOP was evaluated according to Cheson criteria by performing standard hematochemical and instrumental (TC and FDFG-PET) tests and defining complete remission (CR), partial remission (PR), non response or progressive disease (PD). Genomic DNA was extracted from peripheral blood of 80 pts. SNPs analysis was performed by an Affimatrix array. To date, 21 SNPs from 19 candidate genes (ABC1, ABC2, ABC2C, ABC2G, CD80, CD94, FCGR1A, FCGR1B, FCGR2A, GSTP1, IL2, MARG5, MLH1, NCF4, NOQ1, NOQ2, RAC2, TFN, TOP2A, TP53, TUBB) involved in pharmacokinetics and pharmacodynamics of R-CHOP (www.pharmkg.org) selected and analysed in relation to R-CHOP efficacy. Univariate and multivariate logistic regression analyses were performed to evaluate associations between SNPs and clinical and biological characteristics of patients (PFS, OS).

Results: Median age was 63 years. There were 37 men and 43 women. 47.5% of pts were in stage I-II, 52.5% of pts in stage III-IV. 27.5% of pts had bulky disease, 43.8% of pts had involvement of extranodal site. 47.5% of pts had pathological LDH value. According to the revised IPI, 15% of pts were in the low risk group, 58.7% in the intermediate, and 28.3% in the high risk group. The disease was almost always associated with Epstein-Barr virus (EBV), suggesting its role in the etiology of AITL. Neoplastic T cell in most cases are CD4+ and express pan-T cell antigens CD3, CD2, CD5, markers of normal follicular T-helper cells – CD10, CXCL13, PD-1. To confirm the diagnosis and assess disease dissemination combined morphological, immunohistochemical and molecular studies of affected tissues are being used. We have found that T-cell clones detected in the tissue of the lymph node (LN), often differ in T-cell receptor gene rearrangements from those detected in the bone marrow (BM), peripheral blood (PB) and other tissues. T-cell clonality testing itself may not distinguish between neoplastic or reactive lymphoproliferation in the BM and PB. Therefore, T-cell clonality of CD4+ and CD8+ populations of peripheral blood lymphocytes in patients with AITL had been tested during the course of disease.

Aims: To determine immunological characteristics of persisting in the PB and BM T-cell clones in AITL patients.

E1402
CDK4/6-INHIBITION BY ABEMACICLIB INDUCES POTENT EARLY G1-ARREST IN MCL CELL LINES AND SHOWS SEQUENCE-SPECIFIC INTERACTIONS WITH CYTARABINE AND IBRUTINIB L. Fischer1, A. Mayr1, M. Frigger1, B. Freysoild1, Y. Zimmermann1, G. Hutter1, W. Hiddemann1, M. Dreyling1
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Background: Mantle cell lymphoma (MCL) is characterized by t(11;14) resulting in a constitutive cyclin D1 overexpression. The cyclin D1-CDK4/6 complex inactivates Rb through phosphorylation, leading to G1/S-phase transition. Therefore, inhibition of CDK4/6 is an efficient and rational approach to overcome cell cycle dysregulation in MCL.

Aims: We evaluated the efficiency of the novel CDK4/6 inhibitor abemaciclib in various MCL cell lines and in primary MCL cells in combination with cytarabine (AraC) and ibritinib.

Methods: MCL cell lines (Granta 519, JeKo-1, Mav-1, Mino) and primary MCL cells were exposed to abemaciclib alone and combined with AraC or ibritinib. Cell lines were pretreated with abemaciclib and exposed to AraC or ibritinib with or without consecutive wash-out of the CDK4/6 inhibitor. Proliferation and viability were measured by trypan blue staining and Cell Titer Glo assay. Combination Index (CI) to assess synergy or antagonism was calculated using the Fractional Product method by Webb (1963). Flow cytometry was applied for cell-cycle (Pi-staining) and apoptosis analysis (Annexin V/PE/7AAD-staining).

Protein expression and phosphorylation status of various downstream proteins was analyzed by Western Blot analysis.

Results: Abemaciclib inhibited cell proliferation by induction of early G1-arrest. We observed an almost complete and reversible G1-arrest in all sensitive cell lines by FACS analysis (JeKo-1: G1-phase +51.7%; S/G2-phase -51.7% at 31.25 nM after 24 h; G1-phase +35.4%; S/G2-phase -34.8% after 72 h), whereas cell viability was not reduced. IC50-values of sensitive cell lines (JeKo-1: G1-phase +1; G1 +0.24; Mav-1: G1 +0.19; Mino +0.03 for 31.25 nM abe / 2.5 µM ibru), whereas the simultaneous administration of both showed additive effects at most (Cls: JeKo-1 +0.0; Mav-1 +0.1; Mino +0.09 for 31.25 nM abe and 2 µM ibru). In primary MCL cells abemaciclib had no impact on cell death or sensitization since no cell proliferation was observable and cells where resting in G1-phase.

Figure 1.

Methods: The study included 26 patients (15 males and 11 females; age 36-92, median 67) with the diagnosis of AITL established on the basis of WHO 2008 diagnostic criteria. LN, BM and peripheral blood lymphocytes were tested for T-cell clonality according to BIOMED-2 protocol with subsequent fragments analysis on ABI PRISM 3130 (Applied Biosystems). The material was examined at the diagnosis and at various stages of patient’s treatment. In 5 patients selection of CD8+ and CD4+ populations of PB lymphocytes was performed with MidiMACS and miniMACS Separators using CD4+ and CD8+ Microbeads (Miltenyi Biotech), flow cytometry performed for 4 patients in remission with persistent T-cell clones. In all cases, there was a protective effect against AraC treatment in all sensitive cell lines, due to an ongoing G1-arrest (Mino: Cls=0.19 for 31.25 nM abemaciclib / 3.33 µM AraC). Sequential administration of abemaciclib and ibritinib had synergistic or additive effects in sensitive cell lines (Cls: JeKo-1=0.24; Mav-1=0.19; Mino=0.03 for 31.25 nM abe / 2.5 µM ibru), whereas the simultaneous administration of both showed additive effects at most (Cls: JeKo-1=0.0; Mav-1=0.1; Mino=0.09 for 31.25 nM abe and 2 µM ibru). In primary MCL cells abemaciclib had no impact on cell death or sensitization since no cell proliferation was observable and cells where resting in G1-phase.

Summary/Conclusions: The novel CDK4/6 inhibitor abemaciclib causes reversible G1 cell cycle arrest without loss of viability at low nanomolar doses. Rationale drug combinations exploiting the sequential effect may achieve major benefits. Pretreatment with abemaciclib might sensitize cells to ibritinib, resulting in synergistic drug effects. In contrast, simultaneous application of Abemaciclib protects cells from AraC treatment whereas Abemaciclib-induced S-phase synchronization sensitizes MCL cell lines to AraC. Further analysis is needed to explore the interaction with other targeted approaches (inhibitors of the B-cell receptor pathway) to better understand the underlying molecular mechanisms.

E1403
CD8+ T-CELL CLONES PERSISTENT IN BONE MARROW AND PERIPHERAL BLOOD DURING COURSE OF CD4+ ANGIOIMMUNOBLASTIC LYMPHOMA S. Smirnova1, Y. Sidorova1, N. Chernova2, E. Zvonkov2, M. Sinicina3, K. Sychevskaya4, O. Glinishchkova1, N. Ryzhikova1, A. Korvagina3, A. Sudarkov1
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Background: Angioimmunoblastic T-cell lymphoma (AITL) – peripheral T-cell lymphoma, characterized by polymorphous infiltration of the lymph nodes, proliferation of high endothelial venules (HEV) and follicular dendritic cells (FDC). In addition to the lymph nodes, AITL affects spleen, liver, skin and bone marrow. The disease is almost always associated with Epstein-Barr virus (EBV), suggesting its role in the etiology of AITL. Neoplastic T cells in most cases are CD4+ and express pan T-cell antigens CD3, CD2, CD5, markers of normal follicular T-helper cells – CD10, CXCL13, PD-1. To confirm the diagnosis and assess disease dissemination combined morphological, immunohistochemical and molecular studies of affected tissues are being used. We have found that T-cell clones detected in the tissue of the lymph node (LN), often differ in T-cell receptor gene rearrangements from those detected in the bone marrow (BM), peripheral blood (PB) and other tissues. T-cell clonality testing itself may not distinguish between neoplastic or reactive lymphoproliferation in the BM and PB. Therefore, T-cell clonality of CD4+ and CD8+ populations of peripheral blood lymphocytes in patients with AITL had been tested during the course of disease.

Aims: To determine immunological characteristics of persisting in the PB and BM T-cell clones in AITL patients.

Figure 1. Madrid, Spain, June 22 – 25, 2017

haematologica | 2017; 102(s2) | 757
clonal products, which were originally identified in the BM and PB were shown to belong to the CD5+ population of cells (Fig.). In one case BM and PB denuded CD4+ cells also shared a clonal product with LN cells tested at the diagnosis. In one case CD4+ population selected from PB cells at the diagnosis carried clonal rearrangements, fully consistent with that of LN.

Summary/Conclusions: One may conclude that CD5+ T-cell clones identified in PB and/or BM may raise a differential diagnosis of lymphoma. In addition, there are reactive (n=568), during the period 2007-2009. The diagnosis of lymphoma with concomitant HIV infection is, however, challenging as lymphomas may present with high grade B cell non Hodgkin lymphoma (NHL) who were tested for HIV. CD5+ DLBCL showed increased inflammatory markers (C-reactive protein and erythrocyte sedimentation rate) and evidence of increased cell turnover (high uric acid, B2 microglobulin and lactate dehydrogenase levels). Extremely high HIV viral loads (VL) were documented (median 1 612 003 copies/ml, range 12 000 - 10 000 000). Only one patient was virologically suppressed. This is significantly different from lymphoma patients where median VL ranged from 16 000-97 000 dependent on subtype. Median CD4 counts were also higher in this subgroup of patients when compared to patients with lymphoma (see table 1).

E1404

CD5 POSITIVE DIFFUSE LARGE B CELL LYMPHOMA SHOWED FREQUENT MYC EXPRESSION AND AGGRESSIVE CLINICAL BEHAVIOR

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Background: Ablent expression of CD5 distinguishes a unique immunohistochemical subtype of diffuse large B cell lymphoma (DLBCL). This CD5+ DLBCLs, either de novo lesions or transformed from preceding low grade lymphomas or BCL6, may share similar monoclonal B-cell clonality and aggressive behavior. The incidence of CD5+ DLBCL was variably reported from 5-22% of all DLBCLs in western countries and Japan, however, no exact data available in Koreans. Aims: This study aimed to investigate clinicopathologic features of CD5+ DLBCLs.

Methods: A total of 350 cases of DLBCL were reviewed 4 university hospitals from 2004 to 2012. Review of the histologic features along with immunohistochemical-study for BCL1, BCL2, BCL6, CD5, CD10, CD23, IKB, IRF4/MUM1, MYC, Ki-67 and EBV in situ hybridization was performed. Fluorescent in situ hybridization (FISH) for MYC rearrangement and amplification was also performed. The results were compared with DLBCL-NOS (N=195).

Results: Thirty cases of CD5+ DLBCL were retrieved among 350 cases of DLBCL (8.6%), which showed predominance of female (20/30), elderly (mean age 64), and extranodal presentation (16/30). Richter transformation was suspicious in 2 cases and EBV was negative in all. Most cases (22/30) belong to non-GCB subtype by Hans classifier. Rearrangement of MYC was found in 2 cases and amplification was found in one. Compared with DLBCL-NOS, CD5+ cases revealed significantly higher expression of MYC, BCL6, IRF4/MUM1 and Ki-67 (all p<0.05). Double expression of both BCL2 and MYC was found in 9 of 30 cases (30%). Also, CD5+ DLBCL showed more frequent bone marrow involvement, advanced stages and high international prognostic index (all p<0.05). In univariable survival analysis, CD5+ DLBCL revealed significantly shorter progression free survival (median 8.2 months) compared with DLBCL-NOS (median 16.6 months) (p<0.05).

Summary/Conclusions: The clinical, immunophenotypic and prognostic features including cell of origin coincide with previous reports from western population or Japanese. However, frequent high expression of MYC without chromosomal structural alteration was a unique finding in our study. Expression of CD5 should be routinely investigated in DLBCL to find this particularly aggressive subtype.

E1405

REACTIVE FLORID B-LINEAGE LYMPHOPROLIFERATIONS IN HIV INFECTION MAY MIMIC LYMPHOMA

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Background: Approximately 7 million people are living with Human Immunodeficiency Virus (HIV) infection in South Africa (SA) (2015), which is associated with an increased risk of lymphoma. Although there is limited local information available, previously published data from the Johannesburg academic complex of hospitals (SA) showed an HIV prevalence of >90% in patients diagnosed with high grade B cell non Hodgkin lymphoma (NHL) who were tested for HIV (n=568), during the period 2007-2009. The diagnosis of lymphoma with concomitant HIV infection is, however, challenging as lymphomas may present with atypical features and in unusual extranodal sites. In addition, there are reactive conditions which may mimic lymphoma (such as Tuberculosis (TB)). Within this setting reactive florid B-lineage lymphoid proliferations (RBLP) in the blood and bone marrow may raise a differential diagnosis of lymphoma. Aims: This study aims to document the clinicopathological features of florid RBLP in the setting of HIV infection in order to provide an approach to differentiating reactive and clonal processes. Methods: A retrospective database search was performed of the laboratory information system (National Health Laboratory Service) that screened pathology reports for samples referred to the Departments of Molecular Medicine and Haematology and Applied Immunology at the Johannesburg Academic Complex during 2007-2011, supplemented with results of immunophenotypic analysis from 2007-2016. Demographic and clinicopathological findings were collected for patients identified with florid RBLP who showed no definite evidence of monoclonality. Results: During this period, 38 patients were diagnosed with florid RBLP with up to 70-80% of cells in blood or bone marrow comprising reactive B cells (including mature B, plasmablasts and plasma cells). All patients tested were HIV positive, with a median age of 28 years (range 6 months-79 years). There was a bimodal age pattern with a peak in children (<1 year of age (34% of patients) and young adults (18-24 years) (28% of patients). The diagnosis of lymphoma is virtually absent in children under a year of age. Common clinical presentations included cytopenias (85%); infection (70%) (commonly Cytomegalovirus (35%), TB (30%) and bacterial septicaemia (22%); hepatosplenomegaly (42%); and lymphadenopathy (36%). Patients showed increases in serum total protein levels (reflecting hypergammaglobulinemia), with increased inflammatory markers (C-reactive protein and erythrocyte sedimentation rate) and evidence of increased cell turnover (high uric acid, B2 microglobulin and lactate dehydrogenase levels). Extremely high HIV viral loads (VL) were documented (median 1 612 003 copies/ml, range 12 000 - 10 000 000). Only one patient was virologically suppressed. This is significantly different from lymphoma patients where median VL ranged from 16 000-97 000 dependent on subtype. Median CD4 counts were also higher in this subgroup of patients when compared to patients with lymphoma (see table 1).

Summary/Conclusions: In the setting of HIV, reactive conditions may mimic lymphoma and vigilance is needed in the confirmation of monoclonality. Patients with RBLP presented at a younger age when compared to their counterparts with lymphoma. They had extremely high VL with higher CD4 counts, suggesting this may be a feature of early HIV disease and the possibility of a seroconversion type illness should be considered.
Microvessel quantification was performed by immunohistochemical staining, using monoclonal antibodies against platelet/endothelial cell adhesion molecule-CD31. A total of 82 cases of de novo DBLCL treated with R-CHOP were included in the training set for further analysis. There were 45 men and 37 women, with a median age of 57 years (range, 16-84); 35 patients (43%) presented with B symptoms, and 46% (60) had advanced Ann Arbor stages. Most of the patients had a good performance status (Eastern Cooperative Oncology Group score 0-1, 87%), elevated serum lactate dehydrogenase level (61%), and low or low-intermediate International Prognostic Index (IPI) risk (IPI score 0-2, 63%). Involvement of multiple extranodal sites (≥2) was seen in 22% of cases, and bulky disease in 32% of cases.

**Results:** The median follow-up time was 47 months. Among the 82 cases in the training set, CD30 was positive in 24 cases (29%). No difference in response rate was observed between CD30 positive and CD30 negative patients. Patients with CD30+ DLBCL showed a significantly superior OS and DFS compared with CD30− patients. The 5-year OS was 79% in patients with CD30+ vs 59% in CD30− (P<0.05); 5-year PFS was 82% in patients with CD30+ vs 63% in CD30− (P<0.05).

In patients with CD30 positive diffuse large B cell lymphomas we found a smaller number of vessels compared with patients CD30 negative (fig.1, p<0.05).

**E1407**

### ANTIGEN SELECTION PROMOTES CLONAL CYTOTOXIC T-CELL RESPONSES: HIGH-THROUGHPUT IMMUNOGENETIC EVIDENCE

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**Background:** T-cell lymphoproliferations. However, due to the inherent limitations of low-throughput immunogenetic studies, whether there is selection by restricted (perhaps also oligoclonal) antigens remains to be established. That said, earlier, low-throughput immunoprofiling of the clonotypic T cell receptor beta chain (TRBV) genes reflects a dynamic process of cytotoxic T-cell responses against auto- and exogenous antigens remains to be established. That said, earlier, low-throughput immunogenetic studies have implicated antigentic drive in the development of T-LGL leukemias. However, due to the inherent limitations of low-throughput analysis, definitive conclusions were not possible.

**Aims:** To obtain comprehensive insights into the role of antigen selection in the pathogenesis of T-LGL lymphoproliferations using next-generation sequencing (NGS) for in-depth immunoprofiling of the clonotypic T cell receptor beta chain (TRBV) genes.

**Methods:** Included in the study were (i) a father and a son with T-LGL leukemia, the first case of in-famly occurrence; a single blood sample from the father and 2 samples from the son spanning 5 years were analyzed; and, (ii) a patient with T-LGL leukemia of donor cell origin developing after allogeneic hematopoietic cell transplantation (allo-HCT) for Philadelphia-positive acute lymphoblastic leukemia: for this case, the donor blood was analyzed as were two blood samples, one at the first documentation of clonal T-LGL expansion (at 6 months post allo-HCT while investigating persistent neutropenia that developed after Rituximab treatment for EBV reactivation) and a second 3 years later; at both timepoints, the patient had 100% donor chimism and tested negative for BCR-ABL transcripts. TRBV/TRBJ-TRBR rearrangements were amplified on gDNA and subjected to paired end NGS, covering the CDR3 twice/sequence. To increase the consistency of results, raw NGS reads were analyzed by a purpose-built bioinformatics algorithm, performing (i) quality filtering, (ii) merging of filtered in paired reads and (iii) quality filter of stitched sequences. Filtered-in sequences were submitted to IMGT/HighV-QUEST, and metadata was processed by an in-house dedicated bioinformatics pipeline.

**Results:** Only productive TRBV-TRBB-TRBJ rearrangements were included in the analysis. Overall, 1,129,289 filtered-in sequences from 6 samples were evaluated (median 188,095 sequences/sample). Major findings in the familial cases included: (i) prominent T-RBV repertoire of the T-LGL cell consisting of more than one immunodominant clonotype; (ii) in the analysis of longitudinal samples from the son, persisting clonotypes albeit with fluctuating frequencies (clonal drift); and, (iv) shared ('public') clonotypes between father and son. In the T-LGL leukemia of donor origin, the immunodominant clonotype was detected amongst the polyclonal donor repertoire and subsequently expanded in the recipient, persisting over time and accompanied by a few other considerably expanded, albeit smaller, clonotypes.

**Summary/Conclusions:** The borders between polyclonal or oligoclonal versus monoclonal T-LGL lymphoproliferations are not sharply demarcated, but rather, a transition from a polyclonal cytotoxic response to a clonal expansion of more than one immunodominant clonotype; in the analysis of longitudinal samples from the son, persisting clonotypes albeit with fluctuating frequencies (clonal drift); and, (iv) shared ('public') clonotypes between father and son. In the T-LGL leukemia of donor origin, the immunodominant clonotype was detected amongst the polyclonal donor repertoire and subsequently expanded in the recipient, persisting over time and accompanied by a few other considerably expanded, albeit smaller, clonotypes.
samples analyzed by both methods, 83% (139/167) of these were classified as +/+ or -/- by all the FIL labs. The remaining 28/167 (17%) were the samples that showed discordant results in the inter-lab assessments: while in 17 cases the "borderline status" was defined alternatively by only one method, 11 resulted brd samples by both techniques (11/167, 6.6%) (Fig.). Given that the 167 samples were tested in three replicates across the 4 labs, a total of 12 replicates/sample were analyzed: 31 brd samples were thus identified, 13 of which brd by both approaches. Of 156 evaluations performed on the 13 brd, 69/156 (44%) resulted PCR-positive and 87/156 (56%) PCR-negative, 58/156 (37%) were RQ-PNQ and 98/156 (63%) RQ-negative.

Figure 1.

Summary/Conclusions: Despite the high inter-lab reproducibility in the MRD analysis that can be obtained and maintained by the QC round strategy, samples with the lowest MRD levels can still represent a challenge: 17% (28/167) of our series resulted brd, showing discordant results in inter-lab assessments; 39% of them (11/28) remained brd even applying both methods. The results did not change even increasing the number of replicates/sample. Thus, although representing a minority, brd samples are still problematic, especially when a clinically oriented interpretation is required. As the combined use of standard methods does not totally solve this problem, alternative, novel, methods such as digital PCR and NGS need to be tested in this context.

E1409

RHOA GLY17VAL MUTATION AND T-CELL CLONALITY ANALYSIS IN PATIENTS WITH ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA

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Background: Angioimmunoblastic T-cell lymphoma (AITL) is a rare subtype of T-cell lymphoma, characterized by generalized lymphadenopathy, hypergammaglobulinemia, and autoimmune manifestations. Interpretation of histological and immunohistochemical data can be difficult due to the small number of tumor cells in the LN, and brd samples by all the FIL labs are often misdiagnosed as reactive processes and other lymphomas, including Hodgkin’s lymphoma. T cell clonality assessment plays an important role in AITL diagnosis. However, ambiguous clonality results may be obtained. Recently discovered somatic RHOA Gly17Val mutation is present in 53-71% of angioimmunoblastic T-cell lymphomas. We compared the efficacy of T-cell clonality testing and quantitative allele-specific PCR RHOA Gly17Val mutation assay in different tissues for AITL diagnosis.

Aims: To correlate the number of RHOA Gly17Val mutated cells in lymph nodes, blood, bone marrow and skin of AITL patients with corresponding T cell clonality results.

Methods: Lymph nodes (LN), skin biopsies, blood and bone marrow (BM) samples were studied for 40 patients with AITL. The male/female ratio was 25/15, median age was 65 years (36-92). To evaluate T-cell clonality rearranged TCR and TCR gene rearrangements were PCR-amplified according to BIO-MED-2 standardized protocol and analyzed by capillary electrophoresis on ABI PRISM 3130 (Applied Biosystems). Sensitivity of T-cell clonality assay was limited to 10% of clonal T-cells of the total T-lymphocytes in the sample. Gly17Val mutation was analyzed by quantitative allele-specific (qAS) TaqMan Real-Time PCR assay. The detection level of this method was 1% of mutated cells in the total cell population.

Results: The clonal TCR gene rearrangements in LN were found in 37 of 40 patients (92%). RHOA (Gly17Val) mutation in LN was revealed in 60% (24 of 40) patients. T-cell clonality was detected in 26 of 28 primary samples of BM, but in 12 of 26 patients (46%) clonal TCR rearrangements were not matched in length with rearrangements detectable in LN. Number of cells with RHOA mutation was highest in the LN (in average 26.7% of the total cells), while in the bone marrow RHOA mutation was undetectable (in 7 patients), or detected in 10 patients in a small amount (in average 2% of the total cells). Combined historical investigation, T-cell clonality and RHOA (Gly17Val) testing showed BM lesion in 76% of patients (13 of 17) with at least one of the methods. Blood and bone marrow samples examined simultaneously showed slightly higher numbers of RHOA positive cells in the blood than in the BM in 5 of the 7 RHOA positive patients. Significant percentage of cells with a RHOA mutation (in average 25% of the total cells) was revealed in 5 of 6 skin samples from RHOA positive patients. We have found good correlation (Spearman’s Rho=0.8198, p-level <0.00001) between T-cell clonality (matching with LN clonal peaks) and the number of RHOA positive cells in the AITL samples (n 51). Skin, blood and bone marrow samples with the T cell clonality peaks that differ from those found in the LN were also negative for the presence of cells with RHOA (Gly17Val) mutation.

Summary/Conclusions: RHOA (Gly17Val) point mutation is detected in LN by allele-specific PCR in 60% of patients with AITL. The percentage of tumor cells in BM is low (averaging less than 2% of the total cells). However, combined molecular and histological data suggest that BM may be involved in most patients. Extent of T cell clonality (matching with LN clonal peaks) correlates with the amount of cells having a RHOA mutation. T-cell clonality in BM, skin, spleen, etc. with rearrangements not matching those identified for the LN should be considered reactive and possibly associated with autoimmune process or antiviral response.
E1410

USEFULNESS OF CHITOSIDROSIDASE ACTIVITY, CCL18/PARC, 7-KETO-CHOLESTEROL AND GLUCOSYLSPHINGOSINE CONCENTRATIONS FOR SCREENING OF lysosomal storage disorders

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Background: Gaucher (GD), Niemann-Pick Type A/B (NPA/B), Niemann-Pick Type C (NP-C) and Lysosomal acid lipase deficiency (LALD) are lysosomal storage diseases (LSDs) difficult to diagnose due to the great heterogeneity of signs and symptoms, including haematological disorders, sometimes common to several pathologies, and the consequent alteration of biomarkers.

Aims: To assess the diagnostic utility of Chitosidrosidase activity (ChT), CCL18/PARC, 7-ketocholesterol (7KC) and glucosylsphingosine (Lyso-Gb1) concentrations in previously mentioned LSDs.

Methods: ChT activity, CCL18/PARC and 7KC concentrations were measured in 146 plasma samples from subjects with suspected LSD (32 GD, 7 NPA/B, 90 NP-C and 17 LALD) received in our laboratory. In addition, a new biomarker, the Lyso-Gb1 concentration, was evaluated in 83/146 of previous mentioned subjects. 19 of them with confirmed LSD diagnosis. ChT was evaluated using a fluorogenic substrate, CCL18/PARC concentration by ELISA and 7KC and Lyso-Gb1 by liquid chromatography followed by tandem mass spectrometry.

Results: A total of 9/32 (28%) samples with suspected GD showed high ChT and 7KC and 4/9 confirmed GD status: the rest were 1 NPA/B, 1 NP-C and 2 carriers of NP-C. Only 3/43 (7%) with suspected NPA/B and altered biomarkers were confirmed. Among the 23/90 (26%) with suspected NP-C and some elevated biomarker four were diagnosed of NP-C, and two carriers showed some biomarker higher than cutoff. Of the 8/17 (47%) referred to LALD suspicion with some elevated biomarker six were affected. All GD confirmed patients show high levels of Lyso-Gb1 whereas none of the other cases showed elevation for mentioned biomarker.

Summary/Conclusions: The screening of three biomarkers: ChT activity, CCL18/PARC and 7-ketocholesterol concentrations (the latter not applicable in GD) is a powerful tool to identify patients at high risk of suffering from LSDs which should undergo confirmatory diagnostic tests. In this line we would have reduced the number of cases needing confirmatory diagnostic test from 146 to 43 (29%) and 19/43 (44%) were positive for LSDs. Lyso-Gb1 concentration can allow the unambiguous identification of all the GD patients but is not useful for the other LSDs.

E1411

THE VALUE OF SOLUBLE IL-2R ALPHA SUBUNIT MEASUREMENT IN CSF OF CHILDREN WITH HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS (HLH), PRELIMINARY OBSERVATIONS

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Other Non-malignant hematopoietic disorders

Background: Gaucher Disease (GD) is characterized by a latent chronic inflammatory macrophage activation status expressed by an increase of pro-inflammatory cytokines, hyperferrinemia, hypergammaglobulinemia, altered calcium homeostasis and metabolic syndrome. Even patients under ERT do not fully revert this status and their risk to develop bone crises, iron metabolism alterations, autoimmune disorders and neoplasm remain higher. This observation has lead to the creation of a novel marker in GD, lipocalin 2 (Lcn2). Monitoring of patients through chitosidrotidase and CCL18/PARC has become essential however there are patients whom never normalise while others developed bone crisis/ complications after long-time under therapy and normal values. One of the key features for chronic inflammation is the anemia; this is characterized by hyperferrinemia in a common feature described in GD1 patients. Lipocalin (LCN2), a cytokine released by adipocytes, mononuclear cells and neutrophils with expression on endothelial cells, hepatocytes and other cells, has been involved into the monocye polarization and perpetuation of the inflammatory status. Based on this, we have performed an exploratory study assessing LCN2 expression in GD1 patients.

Aims: To explore the Lipocaline (LCN2) expression as biomarker for disease activity in type 1 Gaucher Disease patients under different circumstances.

Methods: We have performed an exploratory study on 18 GD1 patients distributed in two cohorts. Cohort A was composed by 6 patients: 2 naïve (N), 3 patients under miglitol therapy and 1 under miglitol+ERT therapy; this patient was on clinical study QUELAFER and sent from baseline and after 4 months on chelation therapy were obtained. Cohort B included 12 patients on enzymatic replacement therapy (ERT), for this cohort sera samples were obtained for LCN2 determination and also a panel of cytokines (IL-10, IL-13, IL-4, IL-6, IL-7, Mip1a, Mip1b y MCP1), and other parameters, such as ferritin, hepcidin, during the study. Patients were compared at beginning of ERT and after one year on it. Data were incorporated into a database for this porpoise including demographic and clinical available data. All patients have signed an informed consent for the use of their samples and ethical approved were obtained form institutional board of FEETEG foundation. In this study 60% of patients showed LCN2 expression under the cutoff; it was a patients showed increased levels of serum LCN2, the overall mean value for the initial sample was 171, 88 (66,72-261,72). As cohorts the differences among individuals was significant (Cohort A, p=0.02 and cohort B, p<0.01). Naive
patients exhibit the higher values. In general 9 patients showed a reduction on LCN2 levels while 7 showed an increase and one the value was stable. All patients showed a reduction in ferritin and chitotriosidase, however a fully correlation with LC2N expression was not founded. Globally there were no statistically differences, but as individual T-test showed a difference between both measures (p=0.027). A detailed description an analysis will be presented in case of acceptance.

Summary/Conclusions: Lipocaline expression is increased in GO1 patients in general, a correlation with other cytokines expression to establish the role of this biomarker is warranted.

E1413

COMPARISON OF TREATMENT AND OUTCOMES BETWEEN ACQUIRED PRIMARY AND SECONDARY THROMBOTIC THROMBOCYTOPENIC PURPURA

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Background: Thrombotic thrombocytopenic purpura (TTP) is a rare disease that is fatal if untreated. While the main treatment modality is plasmapheresis, immunosuppressants also play a crucial role in the treatment of TTP.

Aims: Our aim is to compare the clinical characteristics, treatment and outcomes of patients with acquired primary TTP to those with secondary TTP (i.e. autoimmune and malignancy/hematopoietic stem cell transplant [HSCT] related).

Methods: We reviewed all patients with TTP who received plasmapheresis at our institution from 1st Jan 2008 to 31st Jan 2017. Clinical and laboratory characteristics, treatment, response to treatment and complications were recorded.

Complete remission (CR) was defined as platelet count normalization, partial remission (PR) as platelet count doubling and <30 x 10⁹/L and the rest as unre sponsive/mortality (UM).

Results: Of 41 cases of TTP, 24% (n=10) was primary, 44% (n=18) was secondary to autoimmune diseases, 27% (n=11) was secondary to malignancy or HSCT. 5% (n=2) was related to DRESS syndrome and acute pancreatitis. The median age was 47 (18-86) years and it was predominately female (81%). About two-thirds of the cases presented with neurological symptoms (66%), renal dysfunction (56%) and fever (59%). Only 12 patients (29%) had TTP pentad.

Proportionate to incidence of secondary TTP, 85% required immunosuppressive therapy and rituximab. Although the final remission rate at end of treatment was 41% (n=17) and at end of follow-up was 46% (n=19), Comparison of demographics, clinical presentation, treatment and outcomes between acquired primary TTP and secondary TTP are shown in table below.

Table 1.

<table>
<thead>
<tr>
<th>Age (median, range)</th>
<th>Primary TTP (n=10)</th>
<th>Acquired immune disease related TTP (n=18)</th>
<th>Maligancy/HSCT related TTP (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(years)</td>
<td>57 (36-66)</td>
<td>64 (46-86)</td>
<td>64 (33-69)</td>
</tr>
<tr>
<td>Female (%)</td>
<td>80 (80)</td>
<td>88 (50-100)</td>
<td>100 (100)</td>
</tr>
<tr>
<td>Neutrophil granulocytes (%)</td>
<td>98 (75)</td>
<td>78 (50-100)</td>
<td>72 (50-90)</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>30 (20)</td>
<td>20 (0-40)</td>
<td>5 (0-10)</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>12.5 (8-18)</td>
<td>11.4 (8-128)</td>
<td>8.2 (4-25)</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>520 (300-1000)</td>
<td>470 (200-1000)</td>
<td>440 (300-700)</td>
</tr>
<tr>
<td>CR (%)</td>
<td>60 (50-100)</td>
<td>68 (50-100)</td>
<td>66 (50-100)</td>
</tr>
<tr>
<td>PR (%)</td>
<td>20 (0-100)</td>
<td>22 (0-100)</td>
<td>22 (0-100)</td>
</tr>
<tr>
<td>LCR (%)</td>
<td>20 (0-100)</td>
<td>22 (0-100)</td>
<td>22 (0-100)</td>
</tr>
<tr>
<td>CR: 30 days, range 15-103)</td>
<td>60 (50-100)</td>
<td>68 (50-100)</td>
<td>66 (50-100)</td>
</tr>
<tr>
<td>PR: 30 days, range 15-103)</td>
<td>20 (0-100)</td>
<td>22 (0-100)</td>
<td>22 (0-100)</td>
</tr>
<tr>
<td>LCR: 30 days, range 15-103)</td>
<td>20 (0-100)</td>
<td>22 (0-100)</td>
<td>22 (0-100)</td>
</tr>
</tbody>
</table>

To evaluate the safety and efficacy of low-dose rituximab combined with corticosteroid treatment in newly diagnosed patients with wAIHA.

Methods: We performed a single-center, prospective, single-arm, open-label study in adult patients with newly diagnosed "primary" or idiopathic wAIHA from 2013-2016 using high-dose dexamethasone (40mg IV days 1-4) followed by 1mg/kg PO daily for 30 days. Prednisone 1mg/kg PO with slow tapering combined with low-dose rituximab. Furthermore, two patients had new-onset immune thrombocytopenia (IT; Fisher-Evans syndrome), without hemolysis. 5 and 6 months after rituximab. Furthermore, two patients had new-onset immune thrombocytopenia (IT; Fisher-Evans syndrome), without hemolysis.

Background: E1415

LOW DOSE RITUXIMAB IS A USEFUL ADDITION TO CORTICOSTEROIDS FOR NEWLY DIAGNOSED PATIENTS WITH WARM AUTOIMMUNE HEMOLYTIC ANEMIA

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Background: Warm autoimmune hemolytic anemia (wAIHA) is an infrequent autoimmune disorder with a high response rate to corticosteroids, albeit relapses are common. Low-dose rituximab has been used successfully in autoimmune hemolytic anemia in an effort to increase response duration, while reducing adverse effects and costs associated with a traditional rituximab dose and prolonged prednisone exposure.

Aims: To evaluate the safety and efficacy of low-dose rituximab combined with corticosteroid treatment in newly diagnosed patients with wAIHA.

Methods: We performed a single-center, prospective, single-arm, open-label study in adult patients with newly diagnosed "primary" or idiopathic wAIHA from 2013-2016 using high-dose dexamethasone (40mg IV days 1-4) followed by 1mg/kg PO daily for 30 days. Prednisone 1mg/kg PO with slow tapering combined with low-dose rituximab (30mg/kg/hour) - one or more times depending on clinical course. Prednisone was administered in 10 (71.4%) patients. All patients received IVIG (1-6g/kg) and high dose methyl-prednisolone (30mg/kg/hour) - one or more times depending on clinical course. Prednisone was administered in 10 (71.4%), cyclosporine in 8 (57.1%) and vincristine in 1 patient. Mean follow-up was 5.4 years (18 months - 13 years), during which 5 (35.7%) patients presented with one or more complications related to treatment: Cushing syndrome, osteopenia, hypertension, renal dysfunction and/or peripheral neuropathy. No severe infection or death was reported during the 15 year period. Disease relapses (1-3) were reported in 8/14 (57.1%) patients.

Summary/Conclusions: The rare entity of Evans syndrome in childhood seems to be associated with various immune manifestations and to carry complications related to treatment. Long term studies are needed to guide optimal management, which still remains challenging.
after diagnosis. Two patients were diagnosed with systemic lupus erythematosus during follow-up, they remained in CR. Twelve-month CR rate was 80% (5 evaluable patients). One patient experienced grade 3 neutropenia two months after the last rituximab infusion that resolved without complications. Estimated relapse-free survival was 80% at 2 years (60% if IT is considered). No patient had a splenectomy performed.

Summary/Conclusions: This small study reports favorable outcomes for patients with newly diagnosed wAIHA treated with low-dose rituximab, and adds 8 patients with similar responses to the 7 cases previously published by the Italian group in 2012 and 2016. These results may be comparable to standard doses of rituximab, with a lower cost, and deserves further inquiry. The emergence of additional autoimmune phenomena (SLE, Evans’ syndrome) is unpredictable and can be an obstacle for appropriate data analysis in prospective AIHA studies.

E1416
INFECTIOUS COMPLICATIONS IN PRIMARY AUTOIMMUNE NEUTOPENIA OF CHILDHOOD
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Background: Primary autoimmune neutropenia (PAN) of childhood is caused by the action of antibodies against membrane antigens of neutrophils leading to their peripheral destruction. Despite the low neutrophil counts, it is characterized by minor intercurrent infections with rare severe bacterial episodes, which can be a significant cause of morbidity.

Aims: The retrospective evaluation of the incidence and characteristics of infectious complications in children with PAN from one reference academic center in Greece.

Methods: The study included the clinical and laboratory findings of children with PAN, who were diagnosed in our department in the last eight years (2008-2016). All children had neutropenia lasting over 3 months with a positive test for neutrophil antibodies, using the granulocyte immunofluorescence test, the granulocyte agglutination test and the monoclonal antibody immobilization of granulocyte antigen test. Laboratory evaluation for nutritional deficiencies, infections, systemic autoimmune diseases or malignancies was negative. Clinical data related to the occurrence of bacterial infections and treatment, hospitalization and outcome were collected and analyzed.

Results: 48 children with PAN were enrolled; 28 were boys, the median age was 14.5 months (range 5-96) and median follow-up time was 20 months (range 4-93). 19 children (39.6%) all suffering from severe neutropenia (<0.5 x 10^9/L) had to be hospitalized 25 times for bacterial infections; 4 for pneumonia, 7 for acute otitis media, 1 for mastoiditis, 7 for urinary tract infections, 4 for bacterial infections of unspecified site, 1 for perianal abscess and 1 for cellulitis, all with good outcome with proper antibiotic treatment. The average number of hospitalizations due to infections was 0.52/patient and the rate was 0.56/1000 patient-days. G-CSF was administered in 2 children due to severe infection, while 8 children received antibiotic chemoprophylaxis.

Summary/Conclusions: Although rare, infections are an important clinical issue in the management of children with severe PAN, sometimes requiring hospitalization. Early signs of infection should be promptly recognized and accordingly treated.

E1417
NEW EPO-RECEPTOR MUTATION IN A -17 YEAR OLD WOMAN WITH ERYTHROCYTOSIS
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Background: Erythrocytosis is defined when red cell, hematocrit (Hct) and hemoglobin (Hb), are elevated above normal limits. Causes of erythrocytosis can be primary and secondary. Secondary causes are divided into congenital and acquired. There is a group of patients with idiopathic erythrocytosis.

Aims: We present a case report of a novel EPO-Receptor mutation.

Methods: We present a case report of a 17-year-old woman with erythrocytosis. In the control blood test she had hemoglobin of 18.6g/dl and hematocrit of 62%. We contacted the patient and she attended hematology consultations for study and treatment with phlebotomy. The patient had no known drug allergies or toxic habits. In 2011 a low oxygen consumption was diagnosed on the basis of low oxygen saturation and a high oxygen requirement for exercise. At evaluation she referred chronic headache without other symptoms. The physical examination was normal. At that time, three possible diagnose were suspected. Firstly, primary erythrocytosis, polycythemia vera (PV). In this disease, the bone marrow produces many red cells and the JAK2 V617F mutation has been demonstrated in the majority of patients. exon 12 mutation has been described in patients with PV who did not have the JAK2 V617F mutation. The erythropoietin (EPO) level is undetectable as a compensatory mechanism. In our patient, JAK2 V617F mutation and exon12 mutation were negative and the EPO levels were undetectable (<1.5). The bone marrow aspirate and the bone marrow biopsy were normal. These results show that this patient doesn’t present PV, due to she only fulfilling one diagnosis criteria of PV. Secondly, acquired secondary erythrocytosis can be produced as a compensatory mechanism, including: cardiac or pulmonary disease, smoking, renal artery stenosis, sleep apnea/hyperventilation and malignant tumors. In the patient, pulmonary function test, abdominal ultrasound and kidney function were normal. Endogenous erythropoietin-receptor mutation. Our patient presented undetectable EPO levels and the EPO-receptor mutation was requested. The patient has been treated with phlebotomies and aspirin due to headache with good evolution. In this moment, she presents hemotocrit levels of 46.8%.

Results: The test revealed an EPO-receptor mutation (c.1275_1290dup), which had never been described before.

Figure 1.

Summary/Conclusions: The study of the patient with erythrocytosis must begin with a full medical history and confirmation of raised Hb and Hct. In the study of erythrocytosis, after ruling out primary and acquired causes we should always consider the possibility of congenital erythrocytosis, which often is underestimated. When EPO binds to its receptor a signaling cascade is activated, which cause red cells to be produced. This process is switched off when sufficient red cells have been produced by binding of SHP-1, EPO-receptor mutation results in failure of bind of SHP-1, causing uncontrolled production of red cells and erythrocytosis. We describe a new EPO-receptor (c.1275_1290dup) (figure 1).

E1418
FAMILIAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN CHILDREN
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Background: Familial hemophagocytic lymphohistiocytosis (FHL) is an autosomal recessive disorder characterized with uncontrolled activation of T-helper lymphocytes and macrophages and over-release of inflammatory cytokines. The only curative treatment is hematopoetic stem cell transplantation (HSCT).

Aims: This study evaluates the clinical and laboratory data of children with FHL. Thirty five FEL cases followed and treated at our clinic between 2005 and 2017 were retrospectively evaluated in our study.

Methods: Information of patients were retrieved from patient files and from the records contained in the electronic information processing environment created after 2005. All patients were treated with HLH-2004 protocol. HSCT was performed in nine patients.

Results: Twenty one of the cases were boys and fourteen were girls. The age at presentation for patients was two week-three years (mean 6.2 months). There was a history of consanguineous marriage in 26 of the families (74%). Fever, anemia, and hypotriglyceridemia were present in all HLH cases. Hepatomegaly was detected in 49.9% of patients. Splenomegaly was more frequent in patients (87.7%). In the majority of the patients, hypofibrinogenemia was detected in the patients. All patients had neutropenia and thrombocytopenia. Hypoferritinemia was present in 94% of patients. Only nine patients found to have hemophagocytosis in the cytological evaluation of the CSF (25.7%). Mutation analysis were performed in all HLH cases. Hepatomegaly was detected in 74%. Splenomegaly was more frequent in patients (87.7%). In the majority of the patients, hypofibrinogenemia was detected in the patients. All patients had neutropenia and thrombocytopenia. Hypoferritinemia was present in 94% of patients. Only nine patients found to have hemophagocytosis in the cytological evaluation of the CSF (25.7%). Mutation analysis were performed in all HLH cases. Hepatomegaly was detected in 74%. Splenomegaly was more frequent in patients (87.7%). In the majority of the patients, hypofibrinogenemia was detected in the patients. All patients had neutropenia and thrombocytopenia. Hypoferritinemia was present in 94% of patients. Only nine patients found to have hemophagocytosis in the cytological evaluation of the CSF (25.7%). Mutation analysis were performed in all HLH cases. Hepatomegaly was detected in 74%. Splenomegaly was more frequent in patients (87.7%). In the majority of the patients, hypofibrinogenemia was detected in the patients. All patients had neutropenia and thrombocytopenia. Hypoferritinemia was present in 94% of patients. Only nine patients found to have hemophagocytosis in the cytological evaluation of the CSF (25.7%). Mutation analysis were performed in all HLH cases. Hepatomegaly was detected in 74%. Splenomegaly was more frequent in patients (87.7%). In the majority of the patients, hypofibrinogenemia was detected in the patients. All patients had neutropenia and thrombocytopenia. Hypoferritinemia was present in 94% of patients. Only nine patients found to have hemophagocytosis in the cytological evaluation of the CSF (25.7%). Mutation analysis were performed in all HLH cases. Hepatomegaly was detected in 74%. Splenomegaly was more frequent in patients (87.7%). In the majority of the patients, hypofibrinogenemia was detected in the patients. All patients had neutropenia and thrombocytopenia. Hypoferritinemia was present in 94% of patients. Only nine patients found to have hemophagocytosis in the cytological evaluation of the CSF (25.7%). Mutation analysis were performed in all HLH cases. Hepatomegaly was detected in 74%. Splenomegaly was more frequent in patients (87.7%). In the majority of the patients, hypofibrinogenemia was detected in the patients. All patients had neutropenia and thrombocytopenia. Hypoferritinemia was present in 94% of patients. Only nine patients found to have hemophagocytosis in the cytological evaluation of the CSF (25.7%). Mutation analysis were performed in all HLH cases. Hepatomegaly was detected in 74%. Splenomegaly was more frequent in patients (87.7%). In the majority of the patients, hypofibrinogenemia was detected in the patients. All patients had neutropenia and thrombocytopenia. Hypoferritinemia was present in 94% of patients. Only nine patients found to have hemophagocytosis in the cytological evaluation of the CSF (25.7%).
ABNORMAL MONOCYTE POPULATIONS IN THE PERIPHERAL BLOOD OF PATIENTS WITH CHRONIC IDIOPATHIC NEUTROPENIA

Aims: Monocyte subpopulations display a prominent role in innate immunity but also mediate pro-inflammatory responses and T-cell activation. The monocyte subset classification in CIN has never been studied before. The aim of the present study was to evaluate the monocyte subsets, namely the classical CD14+/CD16- intermediate CD14+/CD16+ and non-classical CD14+/CD16+ cells as well as the monocyte CD14+/CD15/DRneg/low/CD33+/CD11b+ fraction of the myeloid derived suppressor cells (MDSC), in CIN patients.

Methods: We have studied 25 patients fulfilling the well-defined diagnostic criteria for CIN and 10 age and sex-matched healthy individuals. Three-colour flow cytometry was used to assess the peripheral blood monocytes subsets in the gate of CD14 positive cells and five-colour flow cytometry for the evaluation of the myeloid derived suppressor cells in the gate of cells with intermediate/high FSC/SSC properties.

Results: The mean number of neutrophils and monocytes in CIN patients was 1176±2496/μl and 412±130/μl, respectively. The proportion of classical CD14+/CD16- cells was significantly decreased in CIN patients (79.6%±7.6%) compared to the healthy individuals (87.9%±3.3) (P<0.0009). In contrast, a significant increase was observed (87.90%±3.70%) (P=0.0009). In contrast, a significant increase was observed in the proportion of CD14 positive cells in CIN patients (16.8%±6.75%) compared to the controls (7.97%±3.16%) (P=0.0001). This increase was due to the higher proportion of the intermediate CD14+/CD16+ but not the non-classical CD14+/CD16+ monocyte subset in CIN patients (12.74%±5.28% and 4.05%±2.51%, respectively) compared to controls (7.05%±2.47% and 2.73%±1.39%, respectively) (P=0.0014 and P=0.1383, respectively). Furthermore, the proportion of CD14+/CD15/DRneg/low/CD33+/CD11b+ MDSCs was significantly increased in the patients (6.18%±3.92%) compared to the healthy controls (0.74%) (P=0.0412).

Summary/Conclusions: CIN patients display increased proportion of circulating intermediate CD14+/CD16+ monocytes that may have a role in the aberrant inflammatory responses commonly seen in these patients. The increased proportion of the CD14+/CD15/DRneg/low/CD33+/CD11b+ MDSC in CIN may simply reflect a compensatory reaction aiming to suppress the T-cell activation. Isolation of the above cell populations and transcriptome studies are currently in progress in our laboratory.

References:
Background: Autoimmune hemolytic anemia (AIHA) is not commonly seen in childhood, and is extremely rare in infancy. Absence of guidelines renders management of the disease difficult in children – and even more so in infants.

Aims: Aim of the report is to present a number of cases of infantile AIHA, refractory to conventional treatments, demonstrating response in administration of rituximab.

Methods: The report concerns four infants (3 baby girls and one baby boy) who presented with AIHA. Data including demographics, personal and family medical history, immunologic assessments, previous treatments and response to rituximab were studied.

Results: Age at diagnosis of AIHA was 4-6 months. In 3 cases (cases number 1, 2 and 3) personal and family history, as well as laboratory screening at diagnosis, did not reveal presence of any other hematologic, autoimmune or immunologic condition. In case number 4 AIHA followed the diagnosis of giant cell hepatitis. Hospitalization before rituximab administration ranged between 1 and 11 months due to multiple transfusions, administrative delays and intravenous immunoglobulin (maximum dose 66g/kg), repeated doses of intravenous methyl-prednisolone (30mg/kg) followed by oral prednisolone (max 5mg/kg), all failing to achieve sustained response. Rituximab was administered at 375mg/m² in 4 weekly infusions. In 3 infants 5 monthly infusions followed. Stabilization of hemoglobin and improvement of hemolysis parameters were observed after the 3rd-4th weekly infusion in all infants. In 3 patients (no 1,2,3) CD19+ and CD20+ B cell assessment before and after rituximab administration was performed. Complete elimination (<1%) was observed in all patients after the 1st-2nd infusion. Despite B cells returning to normal 11 months after treatment, infant no 1 remained in clinical remission during follow-up (22 months post treatment). Infant no 2 remained in clinical remission for the 16 month post treatment follow-up, despite B cell normalization. Infant no 3 relapsed following B cell normalization, 11 months after rituximab administration. Infant no 4 did not undergo B cell measurements and relapsed one year after completing rituximab therapy. The 2 patients that relapsed were re-treated with 4 rituximab infusions: patient no 3 remained well for the 18 month follow-up, whereas patient no 4 remained well for 10 years – again relapsing and receiving her 3rd rituximab treatment with good response for the remaining 7 month follow-up. None of the patients presented with adverse reactions during the infusions or with severe infections as a result of immunosuppression. However, in case no 1 Snel developed asymptomatic progressive IgG hypogammaglobulinemia 11 months after initial exposure to rituximab, eventually requiring IVG administration.

Summary/Conclusions: Rituximab administration in refractory AIHA seems to be efficacious and safe in infants. However, close follow-up is warranted in order to ensure absence of long term complications, including the risk of post-treatment hypogammaglobulinemia, when the drug is administered at such young ages.

E1425

CONGENITAL ERYTHROCYTOSIS: DISCOVER OF A NEW MUTATION
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Background: Congenital erythrocytosis (CE) is a rare hereditary disorder of red cell production, characterized by an absolute increase in red cell mass with elevated hematocrit and hemoglobin levels not accompanied by increased EPO production and subsequently in erythropoiesis. EPO production is regulated by Hypoxia Inducible Factor (HIF), 3 prolyl hydroxylase domain proteins (PHD1, PHD2, PHD3), active in the control of EPO synthesis, are able to hydroxylate key prolines in oxygen dependent degradation domains of HIF alpha subunit and it is degraded by the proteasome. Mutations in those proteins are linked to CE.

Aims: Describe a new mutation in PHD2 gene associated to CE.

Methods: Clinical process consultation and search in Blood, European Hematology Association and Pubmed websites of keywords: “congenitalfamilial erythrocytosis” “phd2”.

Results: We described a portuguese family followed by hematology service because of an isolated but sustained erythrocytosis, affecting 3 generations - grandfather, father (propositus) and son. Propositus referred headache and presented plechoric face and hypertension. Analytically, it was confirmed erythrocytosis (haemoglobin>18g/dl and hematocrit>50%), without any other changes, except an indirect hyperbilirubinemia. Secondary causes of erythrocytosis was excluded, with normal EPO and partial oxygen pressure. Bone biopsy only showed an erythroid hyperplasia, no JAK2 mutations identified, and normal hemoglobins electrophoresis, HBB and EPOR gene sequencing. We then proceeded to sequencing of gene included in EPO-induced signaling pathway and it was detected a new mutations in PHD2 gene (F366L), in heterozigosity. Despite it has never been described, other mutations in PHD2 were numerated to slightly increased that can be caused by defects in the EPO-induced signaling pathway (primary), defects in the control of EPO synthesis by the oxygensensing pathway (secondary) or synthesis of high oxygen affinity hemoglobins. EPO transcription is regulated by Hypoxia Inducible Factor (HIF), 3 prolyl hydroxylase domain proteins (PHD1, PHD2, PHD3), active in the control of EPO synthesis, are able to hydroxylate key prolines in oxygen dependent degradation domains of HIF alpha subunit and it is degraded by the proteasome. Mutations in those proteins are linked to CE.

Summary/Conclusions: An unknown mutation of PHD2 has been detected in 2 generation of a family with erythrocytosis and it was co-segregated with the erythrocytosis phenotype. That gene plays an important role in the regulation of EPO production and subsequently in erythropoiesis. Futhermore family studies have to be performed to better understand its pathogeny and management.

E1426

A RETROSPECTIVE STUDY OF THE THROMBOTIC MICROANGIOPATHIES DIAGNOSED IN THE LAST 17 YEARS IN ONE SINGLE CENTRE
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1Hospital Joan XXIII Tarragona, Tarragona, Spain, 2Preventive Medicine Unit, Nefrology, 4Intensive Care Unit, Hospital Joan XXIII Tarragona, Tarragona, Spain

Background: Thrombotic microangiopathies (TMA) are characterized by the formation of platelet thrombi that obstructs vital organ microcirculation. The presence of the 5 classic parameters (haemolytic anemia, thrombocytopenia, fever, oliguria and neurological affection) is rare. ADAMTS13 determination allows a more accurate diagnosis than the presumption based on clinical and biochemical parameters.

Aims: To retrospectively analyze 44 TMA patients diagnosed in our centre in the last 17 years and characterize TTP, HUS and secondary TMA (STMA) by means of clinical, biochemical, laboratory, and flow cytometric data. To correlate with ADAMTS13 level and identify predictors for survival and relapse.

Methods: TMA was defined as microangiopathic hemolytic anemia with thrombocytopenia under 150x10^9/L. All cases were classified as: 1. TTP (TMA with...
ADAMTS13 <5% or TMA without baseline cause). 2. HUS (TMA with ADAMTS13 <5% and high creatinine level and proteinuria, E. Coili Shiga-Toxin or HUS related mutation). 3. sTMA (other TMA with a definite triggering cause).

Clinical and laboratory parameters were analyzed in each group (TTP/HUS/sTMA) (ADAMTS13 ≤5% or >5%) by univariate analysis using chi-square for categorical variables and ANOVA test for continuous variables. Kaplan-Meier and multivariate Cox proportional hazards regression was used for survival and relapse.

Table 1.

Results: Patient distribution was: TTP 13, HUS 8, sTMA 23. ADAMTS13 was determined in 28 patients (low 8, high 20). Clinical and laboratory parameters of each group and univariate analysis are summarised in table 1. All patients received 1mg/kg/day steroids on admission and started plasma exchange. Patients in the TTP group showed increased levels of LDH, schistocytes, bilirubin, and low platelet count which was associated with bleeding. They also required a higher number of plasma exchanges to recover. Five patients relapsed, 4 with low ADAMTS13 level. 4 patients were splenectomized and received immunomodulators. One patient received only plasma exchanges when relapsed. One patient died immediately after diagnosis before receiving plasma exchange. HUS group patients had higher creatinine level which was associated with oliguria and dialysis requirement. Neurological symptoms were more frequent as well. Two patients progressed to renal failure and one was transplanted. Two other patients received eculizumab and 1 relapsed when treatment was interrupted during pregnancy. sTMA patients showed more cardiac events and fever. Main triggering causes were: 6 malignant hypertension, 5 systemic lupus erythematosus, 4 neoplasia, 3 pancreatitis, 2 pregnancy, 1 tuberculosis, 1 glomerulonephritis, 1 dermatomyositis. Six patients died (4 cancer related). In the multivariate analysis, high LDH level was significantly associated with relapse (p=0.012) while the number of schistocytes showed a trend to statistical significance (p=0.063).

Summary/Conclusions: ADAMTS13 determination is a useful tool in TMA differential diagnosis. A high LDH level, and also probably the number of schistocytes, could be valuable to predict relapse in TMA patients.

E1427

CHILDREN WITH CHRONIC-REFRACTORY AUTOIMMUNE CYTOPENIAS: A SINGLE CENTER EXPERIENCE

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1Pediatric Hematology, 2Pediatric Oncology, 3Pediatric Nephrology, Dr. Behcet Uz Children Training and Research Hospital, Izmir, Turkey

Background: Autoimmune cytopenias are a group of heterogeneous disorders characterized by immune-mediated destruction of one or more hematopoietic lineage cells. They can be idiopathic or occur as a manifestation of other underlying disorders, such as autoimmune diseases, immunodeficiency, autoimmune lymphoproliferative syndrome, tumors, medications or infections.

Aims: The aim of this study was to evaluate the clinical course and significance of autoimmune cytopenias due to immunodeficiency or autoimmune diseases in children followed up at our hospital.

Methods: A total of 337 files of information belong to patients with chronic or refractory autoimmune disorders were evaluated retrospectively at our hematology department between February 1997 and September 2015. Ultimately, patients with immune deficiency or autoimmune diseases (23 patients) were included in this study. Data were analyzed using SPSS 15.0. The results are presented as the mean, SD, median, absolute number, or percentile.

Results: Two thirds of the patients with chronic autoimmune cytopenias (6.8%) had an immune deficiency or an autoimmune disease. The median age of diagnosis was 3.1 years (between 6 months-16 years) and the ratio of male/female was 1.3. The median duration of following was 2.6 years (between 4 months and 18.5 years). A total of 13 patients (56.5%) had single-lineage cytopenias and 10 (46.5%) had multi-lineage cytopenias. Shows last diagnoses of the patients. In 5 of the patients, first cytopenias had been more than the primary diseases were diagnosed after median 2 months (between 0 and 77 months). Only one patient firstly had been diagnosed as CVID, cytopenia has developed after years. All of the patients were treated with corticosteroids or intravenous immune globulin (IVIG) as first-line treatment. Ten patients needed second or further-line immunsuppressive therapies including rituximab, cyclophosphamide, azathioprine, and hydroxychloroquine. A total of 8 patients (34.7%) recovered from autoimmune cytopenias after the treatment of primer disease. That diseases were diagnosed as systemic lupus erythematosus in 4 patients, hypogammaglobulinemia in 3 patients, and celiac disease in 1 patient. Cytopenias have been cured in 14 of the patients. One patient with CVID died.

Summary/Conclusions: Cytopenia may be the first finding of an immunodeficiency or autoimmune disease and primary disease may be diagnosed in the clinical course. Early diagnosis is important because of beginning to the early treatment of underlying disease.

E1428

INHERITED PROTHROMBOTIC RISK FACTORS IN TURKISH CHILDREN WITH HEREDITARY ANGIOEDEMA. SINGLE CENTER

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Background: Hereditary angioedema (HA) is characterized with recurrent mucocutaneous angioedema, abdominal pain, edema of larynx and extremities. HA is a life threatening, rare disease, it’s genetic inheritance known as autosomal dominant. The incidence of the disease ranges from 1/10000 to 1/150000. 3 types of disease were described. Classic HA, is associated with C1 esterase inhibitor quantitative (type 1) or functional (type 2) deficiency. Type 3 HA is caused as form of HA which is seen in pregnant women and women use estrogen treatment. If plasma C1 inhibitor is deficient, complement, kinin-bradykinin, coagulation and fibrinolytic systems activate out of control and then vascular permeability increases and angioedema develops, tendency to thrombosis increases as well. Furthermore, it is known that acute treatment with C1 inhibitor prevents acute attacks and prophylactic use of C1 inhibitor may also stimulate the thromboembolism. Therefore, prothrombotic risk factors are important in the patients with HA. Hence, we planned to search prothrombotic risk factors in patients with HA.

Aims: Hence, we planned to search prothrombotic risk factors in patients with HA. Methods: Ten patients with HA who were followed up at the Department of Pediatric Immunology and Allergy of the Erciyes University Medical Faculty were included in our study. The type and frequency of attack, use of prophylaxis and family story of HA were questioned. Factor V G1691A, prothrombin G20210A variant, methylenetetrahydrofolate reductase (MTHFR) and plasminogen activator inhibitor (PAI) mutations were investigated in all patients. Results: During the 10 years of the study, five of the patients had severe attacks (50%) and five were female (50%) and their ages mean was 151,90±48,21 months old (ranged from 75 to 210 months). No one had parental consanguinity. Nine patients (90%) had the family history of HA. Patients’ affected family members were distributed by 5 sibling (50%), 3 mother and aunt (30%), 1 great aunt and father (10%). One patient had no family story (10%). The mean serum value of C4 level in diagnosis was 4,71±1,62mg/dl (normal value: ) mean value of C1 inhibitor level in diagnosis was 50,10±19,22mg/dl (normal value). It was learned that four patients (40%) had an attack of HA once every week, three patients had (30%) once per month, one patient (10%) had, once every 2-3 months. Two patients (20%) had no attack. Four patient had abdominal (40%), four patient had edema of hands, feet and face (40%). None of them received prophylactic treatment. One patient (10%) had heterozygous F V G1691A mutation, another one had also heterozygous prothrombin G20210A mutation. The heterozygous MTHFR mutation were identified in seven patients (70%) and homozygous MTHFR mutation were found two patients (20%). Furthermore, four patients (40%) had heterozygous and one patient (10%) had homozygous PAI mutation.

Summary/Conclusions: C1 inhibitor, inhibits activated F XII,thrombin and plasmin. When the C1 inhibitor is deficient, dermal vascular thrombosis and systemic coagulation occur due to inhibition of activated FXII, thrombin and plasmin. Decrease level of PAI1 and PAI2, destructs plasin activation which is involved on fibrinolysis. Therefore increase tendency of thrombosis and HA risk. In the literature, an adult patient who had heterozygous Factor V leiden mutation and purpura fulminans was reported. In our study, there is no clinical evidence supporting thrombosis, nevertheless it was observed that one of our patient with a homozygous PAI mutation had developed an attack recently. As a conclusion, prothrombotic risk factors should be investigate in patients with HA. In HA patients, known to prothrombotic risk factors was crucial to estimate attack frequency-severity and treatment related thrombosis risk.
Results: Paired BM samples were available. The purpose of this study. Bone marrow samples were obtained from 31 patients (BM) and other tissue samples from patients with hematological malignancies (hM) who developed HLH. The study was intended to investigate potential utility of a rapid phenotypic screening in diagnostics of suspected HLH.

Methods: Flow cytometric files for 42 patients with hM were retrieved from archive of the Department of Clinical Pathology and Cytology, Karolinska University Hospital. The patients were diagnosed and treated for hM-HLH at the Hematology Center of the same hospital, between 2009 and 2016. Tissue samples (bone marrow, peripheral blood, lymph nodes) were analyzed according to standard procedures, using monoclonal antibodies (BD, DAKO, Beckman Coulter, BioLegend). Cells were acquired using 4-color Canto A or 8-color Canto II cytometers (BD), and analyzed with BD FACSDIVA software. Neoplastic clones of myeloid or lymphoid character were excluded from reanalysis for the purpose of this study. Bone marrow samples were obtained from 31 patients shortly before and from 24 patients following HLH-diagnosis; in 13 patients paired BM samples were available.

Results: Patient characteristics are presented in table 1. Bone marrow B-cell lymphophasia was observed in 67% patients before and 74% after HLH diagnosis. Decreased amounts of NK-cells were noted in 48% persons at both time points. T-cell lymphophasia before HLH diagnosis was noted in 60% patients with myeloid malignancy but in only 25% cases of lymphoid malignancy, whereas in established HLH the respective figures were 27% and 46%. CD4/CD8 ratio was skewed-to-normal in both myeloid and lymphatic tumors before HLH diagnosis was diagnosed. In cases of confirmed hyperinflammation, patients with myeloid malignancy showed dominance of CD4+ cells but no such difference was noted in lymphoid disease. Loss of lineage specific markers of non-neoplastic T-cells was a constant feature in lymphoid malignancy, whereas aberrant expression of lymphatic markers (CD2, CD7, CD56) on myeloid cells was uniform in patients with myeloid tumors. Monocytosis was more often observed in myeloid as compared to lymphoid tumors at HLH onset (40% vs 31%), although it was of non-neoplastic character. However, monocytosis was also noted in cases of established HLH, in 10% of myeloid malignancies and 15% of lymphatic malignancy cases.

Table 1.

Summary/Conclusions: In the presented cohort, quantitative shifts could be observed in BM samples around the time of HLH onset. However, different patterns were observed between patients affected by lymphoid or myeloid malignancies, which suggests a shared disease-specific impact on BM microenvironment. Further study will be carried out to confirm findings in a large, possibly prospectively collected patient group. Control group of patients with respective malignancies but without HLH will be included.

Platelets disorders

E1430
BLEEDING IN PRIMARY IMMUNE THROMBOCYTOPENIA: WHO ARE MOST AT RISK?
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Background: Primary Immune Thrombocytopenia is rare disorder in which patients are at risk of bleeding due to autoimmune-mediated platelet destruction. The aim of this study was to identify potential utility of a rapid phenotypic screening in diagnostics of suspected HLH.

Methods: Flow cytometric files for 42 patients with hM were retrieved from archive of the Department of Clinical Pathology and Cytology, Karolinska University Hospital. The patients were diagnosed and treated for hM-HLH at the Hematology Center of the same hospital, between 2009 and 2016. Tissue samples (bone marrow, peripheral blood, lymph nodes) were analyzed according to standard procedures, using monoclonal antibodies (BD, DAKO, Beckman Coulter, BioLegend). Cells were acquired using 4-color Canto A or 8-color Canto II cytometers (BD), and analyzed with BD FACSDIVA software. Neoplastic clones of myeloid or lymphoid character were excluded from reanalysis for the purpose of this study. Bone marrow samples were obtained from 31 patients shortly before and from 24 patients following HLH-diagnosis; in 13 patients paired BM samples were available.

Results: Patient characteristics are presented in table 1. Bone marrow B-cell lymphophasia was observed in 67% patients before and 74% after HLH diagnosis. Decreased amounts of NK-cells were noted in 48% persons at both time points. T-cell lymphophasia before HLH diagnosis was noted in 60% patients with myeloid malignancy but in only 25% cases of lymphoid malignancy, whereas in established HLH the respective figures were 27% and 46%. CD4/CD8 ratio was skewed-to-normal in both myeloid and lymphatic tumors before HLH diagnosis was diagnosed. In cases of confirmed hyperinflammation, patients with myeloid malignancy showed dominance of CD4+ cells but no such difference was noted in lymphoid disease. Loss of lineage specific markers of non-neoplastic T-cells was a constant feature in lymphoid malignancy, whereas aberrant expression of lymphatic markers (CD2, CD7, CD56) on myeloid cells was uniform in patients with myeloid tumors. Monocytosis was more often observed in myeloid as compared to lymphoid tumors at HLH onset (40% vs 31%), although it was of non-neoplastic character. However, monocytosis was also noted in cases of established HLH, in 10% of myeloid malignancies and 15% of lymphatic malignancy cases.

Table 1.

Summary/Conclusions: In the presented cohort, quantitative shifts could be observed in BM samples around the time of HLH onset. However, different patterns were observed between patients affected by lymphoid or myeloid malignancies, which suggests a shared disease-specific impact on BM microenvironment. Further study will be carried out to confirm findings in a large, possibly prospectively collected patient group. Control group of patients with respective malignancies but without HLH will be included.
Patients with platelets <10x10^9/L will commence on eltromobopag 75mg daily while those with a count ≥10x10^9/L will commence on 50mg/day. A step-up dose is used for subjects of East Asian heritage. The dose of eltromobopag can be progressively increased by 25mg increment every 2 weeks to maximum of 150mg daily (patients of East Asian heritage should have a maximum eltromobopag dose of 100mg daily) if the platelet count remains ≤30x10^9/L or if there is clinically significant bleeding every 2 weeks. The dose can be progressively weaned to zero over the subsequent 6 weeks if clinically appropriate. The primary endpoint was overall response rate (ORR) at week 12, defined as the proportion of patients achieving complete response (CR; platelet >100x10^9/L), partial response (PR; platelet ≥50 x10^9/L) or minor response (MR; platelet ≥30x10^9/L with ≥30% reduction in the dose intensity of concomitant ITP therapy compared with screening). The protocol specified a 1-sided 5% level binomial test of the null hypothesis that ORR at week 12 ≤30% and reporting of a 90% two-sided confidence interval (CI).

Results: Of the 39 patients enrolled, 46% were women, median (Q1, Q3) age was 52 (35, 61) years. Median (Q1, Q3) time from ITP diagnosis to first ITP diagnosis was 2.2 (1.1, 5.4) months, and median (Q1, Q3) screening platelet count was 213 (13.3, 34) x10^9/L. Prior treatments included steroids (95%), IVIG (58%), and immunosuppression (28%). 35 patients (90%) completed 12 weeks of treatment, 4 (10%) discontinued eltromobopag prior to week 12. 3 patients with MDS and/or AML, 17 patients had ITP only and 19 patients had other hematological malignancies. The ORR at week 12 was 50 (50, 100) mg daily. The median (Q1, Q3) dose of eltromobopag at week 12, zero (0, 5) mg daily. At week 12, the ORR was 64% (p<0.0001; 90% CI: 51.77%); CR, PR, MR rates were 41%, 15% and 8% respectively and the median (Q1, Q3) platelet count among responders was 168 (98, 252)x10^9/L. At week 26, the ORR was 41% (90% CI 34-48%); CR, PR, MR rates were 28%, 21% and 5% respectively. Two patients had serious adverse events (SAEs) with two episodes of venous thromboembolism (one deep vein thrombosis at platelet 97x10^9/L; one pulmonary embolism at platelet 240x10^9/L). Median (Q1, Q3) survival time was 150mg daily (patients of East Asian heritage should have a maximum eltromobopag dose of 100mg daily) if the platelet count remains ≤30x10^9/L or if there is clinically significant bleeding every 2 weeks. The dose can be progressively weaned to zero over the subsequent 6 weeks if clinically appropriate. The primary endpoint was overall response rate (ORR) at week 12, defined as the proportion of patients achieving complete response (CR; platelet >100x10^9/L), partial response (PR; platelet ≥50 x10^9/L) or minor response (MR; platelet ≥30x10^9/L with ≥30% reduction in the dose intensity of concomitant ITP therapy compared with screening). The protocol specified a 1-sided 5% level binomial test of the null hypothesis that ORR at week 12 ≤30% and reporting of a 90% two-sided confidence interval (CI).

Summary/Conclusions: We revealed novel causal RUNX1 mutation in familial thrombocytopenia. Identification of RUNX1 mutation facilitates proper diagnosis of FPD/AML and identification of heterozygous carriers can help detect the <10x10^9/L have a transformation rate of 28% to AML. Recognition of FPD/AML at the age of 50.

Aims: To analyze mutational status of RUNX1 mutation in disease, aim to identify RUNX1 mutations that can be used as disease biomarkers and to explore the potential biological pathways that might be involved in the pathogenesis of ITP. Methods: Exiqon Serum/plasma Focus microRNA PCR panel was used to determine the expression profile of 179 miRNAs in plasma acquired from 8 ITP patients with low platelet count and who failed to respond to various treatment for ITP, and from 8 age- and sex-matched controls. In addition, next generation sequencing (NGS) for miRNAs was performed in 2 pooled plasma samples (pool 1 from 4 ITP patients, and pool 2 from 4 matched controls), on the Illumina NextSeq 500 system. Statistical analyses were performed with the GenEx software and SPSS. Pathway analysis was performed using DIANA-miRPath v3.0 to explore the probable pathways involved in the pathogenesis of ITP.

Results: Comparing the expression profiling from the PCR panel between ITP patients and matched controls, 81 circulating miRNAs were differentially expressed (p<0.05), of those 17 miRNAs had a high statistical significance (p<0.001). The majority of expressed miRNAs in ITP patients who failed to respond to various treatment for ITP, and from 8 age- and sex-matched controls. In addition, next generation sequencing (NGS) for miRNAs was performed in 2 pooled plasma samples (pool 1 from 4 ITP patients, and pool 2 from 4 matched controls), on the Illumina NextSeq 500 system. Statistical analyses were performed with the GenEx software and SPSS. Pathway analysis was performed using DIANA-miRPath v3.0 to explore the probable pathways involved in the pathogenesis of ITP.

Background: MicroRNAs (miRNAs) are small noncoding RNAs involved in regulation of gene expression. Dysregulated expression of miRNAs has been associated with several autoimmune diseases. ITP is an autoimmune disease characterized by isolated thrombocytopenia and increased risk of bleeding. The development of autoantibodies against platelets and megakaryocytes results in increased platelet destruction and insufficient platelet production remains central to the pathophysiology of ITP. Platelets contain high levels of miRNAs and a substantial fraction of circulating miRNAs originates from platelets. Circulating miRNAs are stable and relatively easy to measure and considered as potential disease biomarkers. The role of miRNAs in the pathogenesis of ITP has not been well explored.

Aims: Determine the expression profile of circulating miRNAs in ITP patients in order to identify miRNAs that can be used as disease biomarkers and to explore the potential biological pathways that might be involved in the pathogenesis of ITP.

Methods: Exiqon Serum/plasma Focus microRNA PCR panel was used to determine the expression profile of 179 miRNAs in plasma acquired from 8 ITP patients with low platelet count and who failed to respond to various treatment for ITP, and from 8 age- and sex-matched controls. In addition, next generation sequencing (NGS) for miRNAs was performed in 2 pooled plasma samples (pool 1 from 4 ITP patients, and pool 2 from 4 matched controls), on the Illumina NextSeq 500 system. Statistical analyses were performed with the GenEx software and SPSS. Pathway analysis was performed using DIANA-miRPath v3.0 to explore the probable pathways involved in the pathogenesis of ITP.

Results: Comparing the expression profiling from the PCR panel between ITP patients and matched controls, 81 circulating miRNAs were differentially expressed (p<0.05), of those 17 miRNAs had a high statistical significance (p<0.001). The majority of expressed miRNAs in ITP patients who failed to respond to various treatment for ITP, and from 8 age- and sex-matched controls. In addition, next generation sequencing (NGS) for miRNAs was performed in 2 pooled plasma samples (pool 1 from 4 ITP patients, and pool 2 from 4 matched controls), on the Illumina NextSeq 500 system. Statistical analyses were performed with the GenEx software and SPSS. Pathway analysis was performed using DIANA-miRPath v3.0 to explore the probable pathways involved in the pathogenesis of ITP.
NORDIC COUNTRY PATIENT REGISTRY FOR IMMUNE THROMBOCYTOPENIA (NCPRITP): A COHORT OF PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA IN DENMARK, SWEDEN, AND NORWAY

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Background: Immune thrombocytopenia (ITP) is a rare disease characterized by isolated low platelet counts and an increased tendency to bleed. As yet, there have been no large, multi-country, population-based cohorts established to describe its long-term clinical course and investigate the effectiveness and safety of related therapies.

Aims: To describe the establishment of the NCPRITP and the characteristics of patients enrolled.

Methods: Encompassing Denmark, Norway, and Sweden, the NCPRITP started as a population-based post-authority safety study to assess the long-term safety of romiplostim in treating ITP. It includes patients with prevalent chronic ITP (cITP – ITP lasting >6 months) as of 04/01/2009, 3% of cITP diagnosed from 04/01/2009-12/31/2014, confirmed through medical record review. Since the start of the registry, guidelines have changed to define cITP as ITP lasting >12 months. For consistency, incident cases of ITP for a duration of >6 months will continue to be accrued through 2019. Through linkage of data from national health registries and medical record review, the registry has rich clinical information for all enrolled ITP patients, such as comorbidities (including scores according to the Charlson Comorbidity Index [CCI] – a validated tool developed to predict 1-year mortality), treatments, lab values (e.g., platelet counts), and complete follow-up for several clinical outcomes of interest (e.g., clinically significant bleeding, the need for rescue therapies, and thromboembolic/thrombotic events). Additionally, available bone marrow samples are restained and reexamined for reticulin and collagen content to assess Thiele’s myelofibrosis (MF) grading.

Results: The NCPRITP includes 3,749 patients with confirmed cITP (35% Danish, 51% Swedish, and 14% Norwegian), with a female predominance (58%) and median age of 56 years at cITP diagnosis. Forty-one percent of the cohort was prevalent at study inclusion; 59% represent incident cITP patients. Median follow-up time thus far is 4.3 years. At study enrollment, 24% had a platelet count <50×10⁹/L, 16% were splenectomized, and 41% had at least one previous ITP therapy (mainly oral glucocorticoid steroids). The majority (68%) of the cohort had no underlying conditions included in the CCI at study enrollment, but 8% had a CCI score of 3 or higher, indicating severe comorbidity. Of note, based on hospital diagnoses of specific comorbidities recorded within 5 years before study enrollment, 25% had hypertension, 9% had a history of DM and diabetes, 18% had a history of hypertension. Currently, 718 bone marrow samples from 566 patients have been retrieved.

Summary/Conclusions: The NCPRITP provides an example of how, within the Nordic countries’ uniform health care systems, registries can be established to study the clinical course of rare diseases such as ITP and the safety of drugs used to treat these patients.

E1436

EPIDEMIOLOGICAL STUDY OF PRIMARY IMMUNE THROMBOCYTOPENIA (ITP) IN ADULTS IN RUSSIAN FEDERATION (RESULTS OF REGISTRY OF NATIONAL HEMATOLOGIC ASSOCIATION)

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Background: Immune thrombocytopenia (ITP) is a rare disease. The incidence of ITP is not well estimated in Russia and worldwide. Due to WHO information it varies from 1.6 to 3.9/100 000 person-years in adults. The gender and age-associated results in Russia and abroad are discussed and differ in several investigations.

Aims: Evaluation of the incidence and demographic characteristics of primary immune thrombocytopenia in adults in Russia.

Methods: The data source is the Registry of the patients with primary ITP in Russia (intermediate data during the 2 years period). 1063 adult patients: 254 females (76%) and 809 males (24%) were splenectomized: 52% female and 74% had platelet counts <30×10⁹/L. Twenty-four pts (48%) withdrew early from the study, most commonly because of AE (n=8, 16%), other reasons (n=7, 14%) and lack of efficacy (n=5, 10%). Median exposure duration was 2.3 years (range, 2 to 7.9 years) and mean daily dose was 49.9 (range, 11–75mg/day). Overall, 43 (86%) pts achieved platelets ≥50×10⁹/L without rescue therapy; 37 (74%) achieved platelets ≥50×10⁹/L for ≥50% of assessments; 26 (52%) maintained platelet counts continuously ≥50×10⁹/L for ≥22 weeks (Fig).

Results: Platelet counts), and complete follow-up for several clinical outcomes of interest (including scores according to the Charlson Comorbidity Index [CCI] – a validated tool developed to predict 1-year mortality), treatments, lab values (e.g., platelet counts), and complete follow-up for several clinical outcomes of interest (e.g., clinically significant bleeding, the need for rescue therapies, and thromboembolic/thrombotic events). Additionally, available bone marrow samples are restained and reexamined for reticulin and collagen content to assess Thiele’s myelofibrosis (MF) grading.

Summary/Conclusions: The NCPRITP provides an example of how, within the Nordic countries’ uniform health care systems, registries can be established to study the clinical course of rare diseases such as ITP and the safety of drugs used to treat these patients.

E1437

ELTROMBOPEG (EPAG) FOR THE TREATMENT OF PATIENTS AGED ≥65 YEARS WITH CHRONIC IMMUNE THROMBOCYTOPENIA (CITP): SAFETY AND EFFICACY RESULTS FROM THE EXTEND STUDY

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Background: ITP is an acquired autoimmune disorder characterized by isolation of platelet counts and an increased tendency to bleed. As yet, there have been no large, multi-country, population-based cohorts established to describe its long-term clinical course of rare diseases such as ITP and the safety of drugs used to treat these patients.
while not receiving rescue treatment, was 78 (range, 0–350) weeks. Incidence of bleeding symptoms (WHO grades 1–4) decreased from BL (66%) to 1 y (15%). AEs were reported in 47 (94%) pts, most frequently nasopharyngitis (n=13, 26%), constipation (n=12, 24%), fatigue (n=12, 24%), diarrhea, arthralgia, urinary tract infection, cataract and cough (all n=11, 22%). Serious AEs occurred in 24 (48%) pts, most frequently (>5%) cataracts (n=7, 14%), pneumonia (n=4, 8%). A total of eight trials including 834 participants were included in the analysis. There was no significant difference of grade 3 or higher adverse events between placebo and treatment group (OR=1.01, CI 0.57–1.78). Thromboembolism (OR=0.59 CI 0.20–1.73), elevated ALT (OR=0.68 CI 0.26–1.74), headache (OR=1.6 CI 0.90–2.78), hypertension (OR=0.2 CI 0.1–0.4), nausea (OR=0.82 CI 0.43–1.55), fatigue (OR=1.13 CI 0.65–1.91) did not show a significant difference between groups, either. Clinical response, which is defined as platelets ≥50,000/μL at least once on treatment, was significantly better in treatment group than in placebo group (OR=0.10 CI 0.07–0.15). Bleeding symptoms (WHO Grades 1–4) were significantly more frequent in the placebo group (OR=1.6 CI 1.14–2.24) during treatment.

Summary/Conclusions: Although several studies have suggested clinically significant treatment-related adverse events, such as thromboembolism, this meta-analysis showed that thrombopoietin receptor agonists are safe, well-tolerated, and effective in patients with previously treated chronic ITP.

E1439

CHILDHOOD IMMUNE THROMBOCYTOPENIA: A NATIONWIDE COHORT STUDY ON CONDITION MANAGEMENT AND OUTCOMES

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Background: Little is known about the management of pediatric ITP in real life, that is, routine clinical practice. Moreover, the predictive value of these factors upon disease outcome was explored individually and therefore the confounding effect of associated exposures remains unknown.

Aims: With this nationwide prospective cohort study, our objectives were to explore (1) the factors associated with treatment initiation (vs. watchful waiting) in children with primary immune thrombocytopenia (ITP) followed in routine clinical practice and (2) the predictors of chronicity at 12 months.

Methods: Between 2008 and 2013, 23 centers throughout France consecutively included 257 children aged 6 months to 18 years and diagnosed with primary ITP over a 5-year period. Data on ITP clinical features along with medical management were collected at baseline and 12 months. Multivariate logistic regressions were used to determine (1) and (2) as defined above, providing odds ratio (OR) with 95% confidence intervals (95%CI).

Results: 137 (53%) children were males, median age 4.6 years, median platelet count was 7×109/L, and 214 (81%) patients initiated medication. Factors independently associated with treatment initiation included platelet counts <10×109/L (p<0.0001) and mucocutaneous bleeding symptoms at baseline (p<0.001). At 12 months, data were available in 211 (82%) children, of whom 130 (74%) had recovered. Predictors of chronicity included female gender (OR=2.2; 95% CI=1.0–4.8), age ≥10 years (OR=2.6; 95% CI=1.1–6.0) and platelet counts ≥10×109/L (OR=3.2; 95% CI=1.5–6.9).

Summary/Conclusions: In routine clinical practice, the decision to apply a watchful-waiting strategy seems to be driven by platelet counts even in the presence of bleeding symptoms, resulting in treatment being initiated in more than 80% of the children surveyed. Overall, younger children with ITP showed good prognosis, with lower platelet counts and, to a lesser extent, male gender predicting more favorable outcomes.

E1440

SIROLIMUS FOR THE TREATMENT OF CHILDREN WITH IMMUNE THROMBOCYTOPENIA AND EVANS SYNDROME: A SINGLE CENTRE EXPERIENCE


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Background: The treatment of chronic relapsing immune thrombocytopenia purpura (ITP) is not well established due to the lack of evidence-based data, and is particularly challenging in children who are at risk of severe side-effects secondary to prolonged steroid therapies. Sirolimus has been shown to be effective in patients with ITP secondary to ALPS1 and in very few patients with primary disease or secondary to ALPS-like syndromes2.

Aims: The aim of this study is to evaluate the outcome and toxicity of patients with ITP either primary or secondary to ALPS-like syndromes, with or without treatment of other underlying diseases.

Methods: We retrospectively evaluated charts of patients followed in our Unit for ITP primary or secondary to ALPS-like syndromes. Patients with ALPS were excluded. ALPS-like was defined as the presence of at least one absolute or primary additional criterion for ALPS. Complete response (CR) and partial response (PR) were defined as a platelet count >100×109/L and >30×109/L and at least 2 fold increase of the baseline count, respectively.

Results: 23 children aged 0.12 yrs (median 6) with primary ITP (7) or secondary to an ALPS-like disorder (16), were treated with Sirolimus. Seven patients (30%) with ALPS-like also had an Evans syndrome (ES), due to the association of leukopenia (1), or to the presence of trilinear cytopenia (6). Four patients with ALPS-like were found to have mutations on PIK3CD, CTTL4, TACI, and CARD 11 gene. All patients, but one treated in first-line, received Sirolimus as second (4), third (14) or fourth (4) line treatment, respectively. 18 patients had previously failed Ficuslenolametofenine (MF) therapy. Overall, 17/23 (74%) patients achieved a response that was complete and partial in 12 (52%) and 5 (21%) patients, respectively. Patients with ES responded in 6/7 (86%) cases. Children with mono-linear ITP achieved a response in 11/16 (68%) cases, in particular 4/7 (57%) and 7/9 (77%) patients with primitive or secondary disease, respectively. 12 out of 18 (66%) patients who failed MF therapy responded to Sirolimus rescue. Three patients (13%) reported toxicity consisting of ory cysts (2) and gastrointestinal issues (1) that required the interruption of the treatment in 2 cases.

Summary/Conclusions: To the best of our knowledge this is the largest cohort of patients with ITP or ES other than -alps treated with Sirolimus, that showed to be safe and effective in most cases, including patients who previously failed
MMF treatment. Therefore, it can be considered as an alternative therapeutic option in the setting of ITP non only for patients with an underlying diagnosis of ALPS but also for the ones with primitive disease or with an ALPS-like disorder.

References

MATERIALS: A CROSS-SECTIONAL STUDY WITH IMMUNE THROMBOCYTOPENIC PURPURA AND CAREGIVERS FOLLOWING RECEIPT OF HOME ADMINISTRATION TRAINING (HAT) MATERIALS: A CROSS-SECTIONAL STUDY

Methods: This non-interventional, cross-sectional study enrolled 40 patients/caregivers and was conducted at 12 centres in Austria, Belgium, France, Germany, Greece, The Netherlands, Spain, and The United Kingdom, from 7 July 2014 to 20 November 2015. HCPs directly observed adults (≥18 years of age) with chronic ITP or caregivers new to administering romiplostim in the act of product administration at the first standard-of-care (SoC) 4-week visit after HAT pack training. Correct administration of romiplostim (primary endpoint) was defined as dose accuracy within 10% margin of error between prescribed and administered romiplostim doses, and correct romiplostim reconstitution and successful injection, and no HCP intervention during administration to correct patient/caregiver error. All analyses were descriptive and no formal hypothesis was tested.

Aims: To estimate the proportion of adult patients and caregivers who administered romiplostim correctly after HAT pack training.

Results: At the first SoC visit, 4 weeks (range: 2-8 weeks) after HAT pack training, 35 patients/caregivers (87.5%) administered romiplostim correctly. The dose accuracy was within 10% margin of error for all patients. HCP intervention was required in 5 instances: 1 patient did not ensure all romiplostim was dissolved, 1 patient and 1 caregiver needed verbal encouragement, 1 patient needed nursing intervention to read the correct dose from the vial due to poor eyesight, and 1 caregiver needed guidance with syringe and vial connection. Further follow-up data was available for only 2 of these 5 patients/caregivers; they both administered romiplostim correctly at a voluntary subsequent visit.

Summary/Conclusions: Given that this study was conducted on a convenience instead of random sample of patients, generalizability of the results may be limited. Direct observation can be susceptible to observation bias and to the Hawthorne effect with the patients/caregivers acting differently when observed. Nonetheless, the success of most patients and caregivers in correctly administering romiplostim after HAT pack training suggests that self-administration of romiplostim is a feasible option for suitable romiplostim-treated ITP patients.

E1442
FCγIIA 131 H/R (A>G) RECEPTOR GENE POLYMORPHISM IN PATIENTS OF PRIMARY IMMUNE THROMBOCYTOPENIA (ITP)

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Background: Primary Immune Thrombocytopenia (ITP) is an autoimmune hematologic disorder characterized by isolated thrombocytopenia (<100,000/mcml) in the absence of other causes or disorders that may be associated with thrombocytopenia. The predominant mechanism is enhanced peripheral destruction of autoantibody coated platelets through binding of Fc portion of antibody with the Fcγ receptors on cells of reticuloendothelial system mainly monocytes/macrophages.

Aims: This study was aimed to investigate the association of polymorphisms in FCγIIA 131 H/R (A>G) with Primary Immune thrombocytopenia (ITP).

Methods: Genotyping for the FCγIIA 131 H/R (A>G) was performed using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) in 70 ITP patients and 70 healthy controls.

Results: The mean age of patients and control was 29.53± 13.86 yrs and 27.90± 8.89 yrs respectively. Male/Female ratio in patients and control was 1:2. Under additive model, the heterozygous genotype (AG) of the FCγIIA 131 H/R (A>G) polymorphism shows the significant association with ITP, (Odds Ratio 2.41 (95% CI, Lower - 1.19 Upper 4.90 P- value 0.0149)) whereas the homozygous mutant genotype (GG) had no significant association with ITP (Odds Ratio 2.47 (95% CI, Lower - 0.63 Upper 9.72 with P- value 0.2976)). Under dominant model, the Odds Ratio was 2.42 (95% CI, Lower - 0.34 Upper 9.94) with the significant P- value 0.0167. Mutant allele (G) frequency was 37.85% in patients and 25.71% in controls (Odds ratio 1.76 1.05-2.93 with the p-value 0.0397).

Summary/Conclusions: The study shows the association of heterozygous genotype (AG) of FCγIIA 131 H/R (A>G) with ITP. The dominant model also shows significant association with ITP. We conclude that mutant allele (G) in FCγIIA 131 H/R (A>G) gene polymorphism may have impact on susceptibility to ITP.
follow-up was 102±9x10^9/L (range 54-336). Disease duration of less than 3 mos prior to therapy start was associated with better outcome (log rank p=0.049, Fig 2) with a median RFS not reached; median RFS for pts treated after 3 mos of diagnosis was 31 mos [OR: 3.8 (CI 95% 0.9-16.3), p=0.067]. No significant association between gender (p=0.57), age at treatment (more or less than 60 yrs) (p=0.85), DTX total dose (more or less than 480mg) (p=0.35) was found. Summary/Conclusions: Pulsed HD-DXM is a well tolerated and highly effective first line treatment for ITP in every day clinical practice. The role of a reduced-dose schedule needs to be explored in a larger cohort of pts. Treatment of newly diagnosed ITP pts - i.e. within 3 mos of diagnosis (Rodeghiero Blood 2007) - seems to lead to longer RFS.

### E1444

**EFFECT OF OSELTAMIVIR TREATMENT ON PLATELET COUNTS**

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**Background:** As platelets lose sialic acid during aging and circulation, they are cleared by the hepatic Ashwell-Morell receptor (AMR) (1). A recent study suggests that inhibition of sialidase by oseltamivir, a commonly administered anti-influenza medication that inhibits viral sialidase, could associate with an increase in platelet counts (2).

**Aims:** The aim of this study was to analyze the effect of oseltamivir treatment in platelet counts.

**Methods:** We performed a retrospective single-center study. From November 2009 until March 2015, a total of 168 patients from our Hematology Unit were prescribed oseltamivir due to clinical suspicion of influenza. A total of 120 patients were excluded because they had received myelotoxic chemotherapy within 30 days (n=82) or platelet count was not available before treatment (n=38). The direct immunofluorescent antigen test was carried out with naphsypharyngeal aspirate specimens. Those specimens that were negative by the antigen detection assay underwent RT-PCR testing for influenza virus types A and B. Platelet count was available before and after treatment (median of 5 days) in 48 patients and in 44 patients also when the infection was cleared (median of 30 days).

**Results:** Patients were divided into those with proven influenza (n=34) and without influenza (n=14). Median age was 58.0 and 59.5 years; respectively. Treatment consisted of 75mg oseltamivir bid for 5 days, with the exception of 3 patients in the proven influenza group receiving 150mg bid for 10 days (allo-geneic stem cell transplant recipients). We observed a significant increase in the mean platelet count after treatment with oseltamivir (170±95 x10^9/L vs 190±103 x10^9/L, p=0.04). As in the previous study (2), this effect was independent of whether influenza was diagnosed (Table 1). In addition, we did not discern significant fluctuation in platelet counts when treatment was immediately interrupted after a 30-day time lapse (184±100 x10^9/L vs 182±91 x10^9/L).

**Summary/Conclusions:** Our study confirms the effect of oseltamivir on increasing platelet counts regardless of influenza infection. Although an increase in platelet counts related to the viral syndrome healing can be expected, the degree and duration of the increase indicates that oseltamivir contributes to reduction in platelet clearance via the hepatic receptor.

**References**


### E1445

**CLINICAL UTILITY OF CARDIAC MRI IN IMMUNE MEDIATED THROMBOCYTOPENIC PURPURA**

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**Background:** Immune Mediated Thrombotic Thrombocytopenic Purpura (TTP) is a life threatening thrombomicroangiopathy caused by acquired antibody mediated inhibition of ADAMTS13. Cardiac complications are a common cause of death in patients who have recognized thrombotic microangiopathy in TTP. There is scant evidence on the best investigations for patients suspected of being at risk of cardiac complications with no evidence on the clinical utility of cardiac magnetic resonance imaging (MRI) in acute TTP episodes.

**Aims:** A retrospective review evaluating the value of cardiac MRI scanning in TTP. Methods: 20 patients underwent cardiac MRI between September 2008 and November 2014 whilst being treated for an acute episode of immune mediated TTP. All patients had troponin-t measurement on admission and a transthoracic echocardiogram within 72 hours of presentation. All patients were treated for their TTP episode with plasmapheresis, steroids and Rituximab. Two cardiologists reported each MRI scan and only agreed, unequivocal findings were considered.

**Results:** The median age of patients was 49 (range 13-73), 71% of whom were women. Two patients had a diagnosis of hypercholesterolemia prior to TTP diagnosis but otherwise there was no previous cardiac history. 71% of patients had a raised troponin-t at presentation (normal <14µg/ml). Two patients developed bradycardia and one atrial fibrillation during their acute admission. One patient had symptoms of heart failure. Three patients had transient ST depression suggestive of ischemia on EKG monitoring and a further four had non-specific T-wave inversion. There were no incidences of cardiogenic shock or STEMI. No patient was cleared of myocardial infarction. 33% of patients had no evidence of cardiac dysfunction. No patient had a normal cardiac MRI (normal MRI 100ng/ml, abnormal MRI 165ng/ml, p=0.9), nor was there a significant difference in median age (49 vs 49), symptom duration (abnormal MRI 7 days, normal 5 days, p=0.39) or presenting anti-ADAMTS13 antibody level (abnormal MRI 41%, normal MRI 40%, p=0.60).

**Summary/Conclusions:** Cardiac MRI scanning in TTP is a sensitive tool for detecting ischemic cardiac changes that would otherwise be missed by transthoracic echocardiogram. Mid-Apical late gadolinium enhancement appears to be a characteristic finding in TTP. These findings help increase the understanding of the pathophysiology behind the TTP disease process.

**References**


**Table 1.**

<table>
<thead>
<tr>
<th>Platelet counts (x10^9/L)</th>
<th>In patients with a clinical suspicion of influenza (median of 5 days)</th>
<th>Results are given as medians (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet counts</td>
<td>In patients with a clinical suspicion of influenza (median of 5 days)</td>
<td>Results are given as medians (range)</td>
</tr>
<tr>
<td>Infection positive</td>
<td>Infection negative</td>
<td>Infection positive</td>
</tr>
<tr>
<td>190±103 x10^9/L</td>
<td>184±100 x10^9/L</td>
<td>182±91 x10^9/L</td>
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**References**

group and 56 (24-76) in the control group. Overall MEFV mutation prevalence was 29.3% (21/72) in the study group and 42.4% (74/176) in the control group, (p=0.093). MEFV mutation distribution prevalence was similar in both gender groups among ITP patients and their presence did not alter the age of disease onset, (p<0.05). Similarly, presence of mutations did not change the platelet count at diagnosis, the number of treatment courses, the rate of patients undergoing splenectomy and primary steroid resistance. Although statistically not significant, there was a trend towards a better overall response to steroids in patients carrying MEFV mutations, 94.7% vs 82.8% (p=0.28) respectively. The median time to loss of response to steroids was 60 (10-124) months in patients with mutations and 42 (19.2-64.8) months in patients without MEFV mutations, (p=0.03). The median time to splenectomy was 101 (42.5-159.5) months in the MEFV mutation carriers and 51 (46-56) months in the non-carriers, (p=0.48). Time to loss of response to splenectomy was 38 (12.90.9) months in mutation carriers and 54 (14.9.31) months in non-carriers, (p=0.42).

Summary/Conclusions: To the best of our knowledge, our study is the first to address the role of MEFV mutations on MEFV mutation carrier rates were similar in both ITP and control groups. Although MEFV carrier state had no effect on clinical features of ITP, mutation carriers tended to have a better overall response to steroid treatment, stayed longer in remission, had a longer time to splenectomy and relapsed earlier after splenectomy.

E1447
PD-1 AND CTLA-4 POLYMORPHISMS AFFECT THE SUSCEPTIBILITY AND CLINICAL FEATURES OF CHRONIC IMMUNE THROMBOCYTOPENIA

Background: The programmed death-1 (PD-1) and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) play a central role in immune checkpoint pathways. The PD-1 negatively regulates self-reactive T and B cells in peripheral immune tolerance. The CTLA-4 antagonizes the binding of CD28 to its ligands including CD80 and CD86, and inhibits T cell activation. Previous studies have shown the lower expression of serum soluble PD-1 and CTLA-4 mRNA in patients with chronic immune thrombocytopenia (cITP) than healthy individuals. Single nucleotide polymorphisms (SNPs) of PD-1 and CTLA-4 have been reported to be associated with susceptibility of some autoimmune diseases; however, the possible association between these immune checkpoint SNPs and cITP risk remain controversial and obscure.

Aims: In order to explore the role of PD-1 and CTLA-4 in the pathogenesis of cITP, we investigated the impact of PD-1 and CTLA-4 SNPs on the susceptibility and clinical features of adult cITP.

Methods: We extracted the genomic DNA from 141 cITP patients and 223 healthy controls, and determined 3, PD-1 SNPs (-606G/A, -7209C/T, A215V) and 4, CTLA-4 SNPs (-1577A/G, -1558A/G, -4630G/A, -6230A/G) by using the polymerase chain reaction -restriction fragment length polymorphism (PCR-RFLP) method. The severity of bleeding tendency and thrombocytopenia was assessed according to the previously described criteria by Han J.J. The response criteria, "corticosteroid dependence," severe cITP, and refractory cITP were defined according to the criteria of the ITP International Working Group. The characteristics and laboratory data of cITP patients with PD-1 and PD-1 polymorphisms were compared by using the Mann-Whitney U test for continuous variables and the chi-square test for categorical variables. This study was approved by the Institutional Review Board of Gunma University Hospital (Approval # 160007).

Results: The minimum platelet count of all clinical course ranged from 0 to 98×10^9/L with a median count of 13×10^9/L. Eighty-six patients (61.0%) had bleeding tendency and 24 patients (17.0%) had severe thrombocytopenia (<10×10^9/L). Eighty-six patients (61.0%) received the treatment with corticosteroids and A. 1558G was correlated with 1558C (22.7%) with corticosteroid dependence, higher frequency of PD-1 and CTLA-4 TT genotype (low producer) was observed in cITP patients (12.8% vs 4.5%, p=0.004). There were no significant differences in CTLA-4 SNPs between cITP patients and healthy controls. In cITP patients, PD-1 and CTLA-4 TT genotypes were significantly associated with 23.6% and 32.9% vs 23.6% and 32.9% vs 23.6% (p=0.043, 0.003 and 0.188, respectively). On the other hand, CTLA-4 -49AA genotype (high producer) was significantly associated with lower bleeding tendency than AG & GG genotype (low producer) (27.3% vs 63.8%, p=0.017). CTLA-4 -1577 AA genotypes (high producer) was significantly associated with low bleeding tendency and steroid treatment than AG & GG genotype (low producer) (27.3% vs 63.8%, p=0.017). CTLA-4 -49AA genotype (high producer) was significantly associated with higher platelet count than AG & GG genotype (22.5 vs 14.0×10^9/L, p=0.048). The programmed death-1 (PD-1) and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) play a central role in immune checkpoint pathways. The PD-1 negatively regulates self-reactive T and B cells in peripheral immune tolerance. The CTLA-4 antagonizes the binding of CD28 to its ligands including CD80 and CD86, and inhibits T cell activation. Previous studies have shown the lower expression of serum soluble PD-1 and CTLA-4 mRNA in patients with chronic immune thrombocytopenia (cITP) than healthy individuals. Single nucleotide polymorphisms (SNPs) of PD-1 and CTLA-4 have been reported to be associated with susceptibility of some autoimmune diseases; however, the possible association between these immune checkpoint SNPs and cITP risk remain controversial and obscure.

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E1450
THE CLINICAL UTILITY OF NEUROPSYCHOLOGY TESTING IN IMMUNE MEDIATED THROMBOTIC THROMBOCYTOPENIC PURPURA
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Background: It is well recognized that neurological manifestations are common in thrombotic thrombocytopenic purpura (TTP). However, research into the neuropsychological impact of the disease is scarce, despite potential risk factors including antithrombotic therapy and re-splenectomy. As such, the purpose of this study was to determine the incidence of neuropsychological symptoms in patients with TTP.

Aims: To review the clinical utility of neuropsychology testing in thrombotic thrombocytopenic purpura.

Methods: Between 2010 and 2015, all patients within a single tertiary haematology center with a confirmed diagnosis of TTP were reviewed as outpatients after their acute episode. Those with persisting, non-physical neurological or psychological symptoms underwent cerebral MRI scanning and were referred for neuropsychological assessment. The Wechsler Adult Intelligence Scale (WAIS-III) review was used to assess factors including verbal IQ and performance IQs.

Results: 18 patients were included. 89% were female with a median age of 51 (16-67 years). 56% were Caucasian, 33% Afro-Caribbean and 11% of South Asian ethnic origin. 33% had experienced TIA or stroke-like symptoms during their acute TTP episode whilst 28% had no neurological symptoms during their acute TTP episode. 117 patients (85.4%) were non-splenectomized before romiplostim initiation. The median time from acute TTP episode to neuropsychology review was 29 months (range: 3-99 months). 25% had normal cerebral MRI scans, 50% had mature infarcts and microhaemorrhages. The median scores for both verbal and performance IQs were reduced compared to average (normal 100, range 90-110). The median verbal IQ was 87 (range: 65-122) and the median performance IQ was 83 (range: 56-109). Taking all aspects of the WAIS-III review into consideration, most of them received corticosteroids (104 [75.9%]). 117 patients (85.4%) were non-splenectomized before romiplostim initiation. The most frequent ADRs were gastrointestinal (10.2%) and neurological (11.7%) ADRs, followed by constitutional symptoms (10.9%). Adverse drug reactions pertaining to blood/bone marrow affected 2.9% of patients (vascular/thrombotic events, bone marrow fibrosis), whereas bleeding as an ADR was seen in 0.7% of patients. The exposure-adjusted rate of bleeding events (grade 3 or 4) per 100 patient-years in the FAS was 7.2 before treatment vs 4.0 after starting the treatment. The rate of ITP-related hospitalization per 100 patient-years decreased from 23.3 before the start of therapy to 15.5 since the start of therapy.

Summary/Conclusions: This study of routine clinical practice in Germany showed that treatment with romiplostim in ITP patients resulted in a rapid increase in platelet counts to levels maintained between 50 and 250 x 10^9/L over time, regardless of the splenectomy status of the patients; most of them were non-splenectomized. The product was well tolerated and achieved a decrease in the rate of ITP-related hospitalization.

E1451
FIVE NEW CASES OF HERMANSKY-PUDLAK SYNDROME: IDENTIFICATION OF NOVEL GENETIC VARIANTS IN HPS4 AND HPS3 ASSOCIATED TO RELEVANT CLINICAL COMPLICATIONS
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Background: Hermansky-Pudlak syndrome (HPS) is an inherited platelet disorder characterized by bleeding diathesis, oculocutaneous albinism and some times serious clinical complications. Heterogeneous clinical symptoms and a large numbers of possible genetic culprits (9 HPS genes, >118 exons) complicate unequivocal HPS diagnosis.

Aims: To assess the clinical and platelet phenotype in five patients with HPS suspicion and to identify their genetic defects (through HTS using a 7 gene panel).

Methods: We studied 5 patients from 3 families (2 Spanish, 1 Turkish) presenting with oculocutaneous albinism. Clinical records were reviewed and bleeding scored using ISTH-BAT. Platelet phenotyping (only Spanish patients) included: platelet aggregation, GPIIb expression and granule secretion. 14C-serotonin uptake and whole mount electron microscopy. Patients DNAs were analyzed by HTS using a 7 gene panel.

Results: Clinical and laboratory findings in these patients are shown in Table 1. The Spanish patients (P1, P2, P5) showed impaired platelet aggregation to mild agonists and reduced platelet dense granules. In family 1 (F1), HTS identified a heterozygous, potentially harmful, c.2054delC (p.Pro685Leu fs*17) variant in HPS4. One sister (P1) had Crohn’s disease and severe gastrointestinal (GI) bleeding. This variant had been reported in a 46y Asian patient with pulmonary fibrosis (Bachi EB, Am J Med Genet 2004). A novel missense homoygous HPS4 variant, c.272T>C (p.Leu91Pro), was found in two Turkish siblings (F2). One had severe GI bleeding requiring coloectomy and the other developed pulmonary fibrosis. Patient 5, suffering from mild GI bleeding, bears a heterozygous novel variant in HPS3 (c.2464C>T, p.Arg822X) and, most likely, an additional unrevealed mutation.

Table 1.

Summary/Conclusions: HTS facilitates genetic confirmation of HPS diagnosis, and may help investigating phenotype-genotype relationships in HPS. The novel p.Leu91Pro variant in HPS4 associates with severe clinical phenotype. Funding: JMB: Gerencia Regional de Salud [GRS 1370/A/16]; JR: ISCIII & Feder (PI14/01956), Ciberer CB15/00055, Sociedad Española de Trombosis y Hemostasia.
hemolysis, elevated liver enzymes, and low platelets. Previous studies have demonstrated elevated platelet activation in pregnant women with pre-eclampsia, using cell surface markers and platelet microparticles. Although severe pre-eclampsia is associated with increased inflammatory markers in vitro, levels of platelet activation do not necessarily correlate with severity of disease.

Aims: To assess the presence, and degree, of platelet activation in a cohort of patients with early onset pre-eclampsia (EOP) and HELLP syndrome, and to correlate this with evidence of in vivo coagulation activation using D-Ubers.

Methods: Plasma samples from patients with EOP were accessed from a clinical biobank. Platelet activation markers were characterized using ELISA assays measuring platelet factor 4 (PF4), soluble glycoprotein VI (sGPVI) and neutrophil activating peptide-2 (NAP-2). Platelet microparticles (CD42a+ microparticles) were measured by flow cytometry. Platelet activation biomarker levels were adjusted by platelet count and expressed as /10⁸ platelets/ml. All data was analysed using GraphPad Prism. 7. Parameters were reported as means±SEM.

Results: Plasma samples from 19 individual patients were included. Patients with HELLP syndrome demonstrated significantly greater numbers of CD42a+ microparticles when corrected for platelet count compared with those without HELLP syndrome (598±10³±20x10³ vs 297±10³±37x10³ platelets/ml; p=0.04). Similarly, patients with HELLP syndrome demonstrated increased levels of sGPVI than those without HELLP; corrected for platelet count (2.47±0.56 vs 2.12±0.24 µg/ml; p=0.0334). There was no difference in NAP-2 or PF4 levels between those with HELLP and those without HELLP, nor between severe and moderate pre-eclampsia patients. Severe pre-eclampsia patients in this cohort had a D-dimer level of 3.7±1.01±742 µg/ml compared with non-severe patients 1.85±0.50±351 µg/ml (p=0.0337). There was a significant correlation between sGPVI levels and D-dimer levels (Spearman Rank correlation coefficient, r = -0.532, p=0.04).

Summary/Conclusions: The results of this study demonstrate a positive correlation between severity of pre-eclampsia and platelet activation, as measured by levels of platelet-derived microparticles and platelet GPVI expression. A number of anticoagulant mediated treatments have evaluated the role of low-dose aspirin therapy as prevention for pre-eclampsia, and there is Grade 2B evidence for its use in those at risk of severe pre-eclampsia. The evidence of enhanced platelet activation in our study provides rationale for the efficacy of aspirin in this setting, and the potential for novel antiplatelet agents to be studied for this indication.

E1454

PRIMARY ITP IN ADULTS TREATED WITH ELTROMBOPAG: A RETROSPECTIVE STUDY USING DATA FROM THE UNITED KINGDOM ADULT IMMUNE THROMBOCYTOPENIA REGISTRY.

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Background: Primary ITP is an autoimmune disorder associated with a reduced peripheral blood platelet count. Although many patients are relatively asymptomatic, many suffer with bruising, mucosal bleeding and quality of life issues. As a result many patients receive one or more lines of treatment, including splenectomy. The two currently available agents (Romiplostim and Eltrombopag) have similar efficacies and only slightly different safety profiles, being effective in restoring a safe platelet count in 70%-80% of cases with chronic ITP failing one or more lines of treatment, including splenectomy.

Aims: We evaluated the efficacy of TPO-RAs in patients with ITP.

Methods: From November 2008 and February 2017 65 patients (33 M; 32 F) were treated with a median follow-up of 29 months (1-96): 39 underwent therapy with Romiplostim and Eltrombopag. Median age was 69 years (range 39-94 years). In the group of patients treated with Romiplostim, 21 had already received more than 4 lines of treatment, whereas 18 were treated as first or second line therapy. Thirteen patients received 3 or more prior therapies before starting eltrombopag despite its licence as a second line therapy. Threequarters had received one or more prior ITP therapies and 99 patients (77%) had received three or more prior therapies before starting eltrombopag. The commonest prior therapies were corticosteroids in 110 patients (87%); IVIg 91 patients (72%); rituximab 68 patients (54%); romiplostim 47 patients (37%); and immunosuppressants 71 patients (56%). At baseline, prior to starting eltrombopag, the median platelet count was 21x10⁹/L (10-54) and the majority of patients (64.5%) had platelets less than 30x10⁹/L. The median platelet count at 6 months was 206±2x10⁹/L and at 1 year was 288±10³/L. The median dose of eltrombopag used was 50mg/day. The median course length on eltrombopag was 14.7 (IQR: 4- 67) weeks. After initiation, 53 (41%) remained on eltrombopag as a monotherapy whereas 27 (21%) had other ITP treatment concurrently with eltrombopag. Forty nine (38%) changed treatment after eltrombopag, of which prednisolone (47%), IVIg (35%), monoclonal antibodies (14%) and aspirin therapy (10%) were used. Forty patients (30%) underwent a splenectomy. Response to eltrombopag was assessed for 106 patients with adequate follow up time and platelet counts. 81 (76%) had a response, of which 54 (51%) were above 100x10⁹/L and 27 (25%) had a partial response (platelet counts between 30 to 100x10⁹/L). Among those that had a response, 15 (14%) became unresponsive after some time whereas 2 (2%) patients were unresponsive soon after a brief episode of response. In short, 64 (60%) had a sustained response to eltrombopag (among patients who remained or came off eltrombopag).

Summary/Conclusions: The patient characteristics of those receiving eltrombopag appear to be typical of adult ITP. Only 10 patients (7.8%) had received no prior ITP therapies. The second line therapy. Three quarters had received 3 or more prior therapies before starting eltrombopag despite its licence as a second line therapy. As clinicians become more familiar with its use, a greater proportion of patients are likely to receive eltrombopag as a second line therapy.

E1454

EFFICACY OF TPO-MIMETICS IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA

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Background: Immune thrombocytopenic purpura (ITP) is an autoimmune disorder in which antibodies are produced to circulating platelets. The two currently available agents (Romiplostim and Eltrombopag) have similar efficacies and only slightly different safety profiles, being effective in restoring a safe platelet count in 70%-80% of cases with chronic ITP failing one or more lines of treatment, including splenectomy.

Aims: To assess the efficacy of TPO-RAs in patients with ITP.

Methods: From November 2008 and February 2017 65 patients (33 M; 32 F) were treated with a median follow-up of 29 months (1-96): 39 underwent therapy with Romiplostim and Eltrombopag. Median age was 69 years (range 39-94 years). In the group of patients treated with Romiplostim, 21 had already received more than 4 lines of treatment, whereas 18 were treated as first or second line therapy. Thirteen patients received 3 or more prior therapies before starting eltrombopag despite its licence as a second line therapy. Threequarters had received one or more prior ITP therapies and 99 patients (77%) had received three or more prior therapies before starting eltrombopag. The commonest prior therapies were corticosteroids in 110 patients (87%); IVIg 91 patients (72%); rituximab 68 patients (54%); romiplostim 47 patients (37%); and immunosuppressants 71 patients (56%). At baseline, prior to starting eltrombopag, the median platelet count was 21x10⁹/L (10-54) and the majority of patients (64.5%) had platelets less than 30x10⁹/L. The median platelet count at 6 months was 206±2x10⁹/L and at 1 year was 288±10³/L. The median dose of eltrombopag used was 50mg/day. The median course length on eltrombopag was 14.7 (IQR: 4- 67) weeks. After initiation, 53 (41%) remained on eltrombopag as a monotherapy whereas 27 (21%) had other ITP treatment concurrently with eltrombopag. Forty nine (38%) changed treatment after eltrombopag, of which prednisolone (47%), IVIg (35%), monoclonal antibodies (14%) and aspirin therapy (10%) were used. Forty patients (30%) underwent a splenectomy. Response to eltrombopag was assessed for 106 patients with adequate follow up time and platelet counts. 81 (76%) had a response, of which 54 (51%) were above 100x10⁹/L and 27 (25%) had a partial response (platelet counts between 30 to 100x10⁹/L). Among those that had a response, 15 (14%) became unresponsive after some time whereas 2 (2%) patients were unresponsive soon after a brief episode of response. In short, 64 (60%) had a sustained response to eltrombopag (among patients who remained or came off eltrombopag).

Summary/Conclusions: The patient characteristics of those receiving eltrombopag appear to be typical of adult ITP. Only 10 patients (7.8%) had received no prior ITP therapies. The second line therapy. Three quarters had received 3 or more prior therapies before starting eltrombopag despite its licence as a second line therapy. As clinicians become more familiar with its use, a greater proportion of patients are likely to receive eltrombopag as a second line therapy.

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PREVALENCE AND RISK FACTORS FOR THROMBOSIS IN ADULT ITP PATIENTS
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Background: Immune thrombocytopenia (ITP) is characterized by severe thrombocytopenia due to autoantibody- and cell-mediated peripheral platelet destruction and attenuated thrombopoiesis. Despite a higher risk for bleeding, thromboembolic events (TEE) have been observed.

Aims: We aimed to investigate the prevalence and type of TEE and the potential risk factors in adult ITP patients.

Methods: Retrospective cohort study, including all ITP patients followed in our clinic between 01/1990 and 05/2016. Information on gender, age, date of ITP diagnosis, platelets count, type and clinical form of ITP, type of ITP treatments and its response, severe bleeding and follow up time were collected. Furthermore we evaluated date of first appearance, number and type of thromboembolic events, cardiovascular risk factors, date and cause of death. We assessed and compared risk factors of ITP patients with and without TEE in univariate and multivariate analysis.

Results: Medical files of 480 patients registered as ITP were reviewed; 42 patients were excluded from the analysis (not fulfilling the ITP criteria according to Rodgheiro et al. Blood 2009). In total 438 patients were retained for analysis, 10% out of them (44 patients) presented ≥1 TEE after ITP diagnosis. Within these patients, in total 54 TEE occurred: 34 venous (61%), 19 arterial (34%) and 3 arterial and venous (5%) thrombotic events. The most frequent venous TEE were pulmonary embolism, deep vein thrombosis, and superficial vein thrombosis; arterial TEE were cerebrovascular insults, myocardial infarction and peripheral artery thrombosis. At time of TEE, 43% of patients were on treatment with corticosteroids, 14% with thrombopoietin receptor agonists (TPO-ra) and 18% were off-treatment. In the univariate analysis, older age at diagnosis (<50 vs ≥50 years, P=0.015), longer interval since ITP diagnosis (P=0.009), ≥2 treatment lines (P=0.0002), TPO-ra at time of thrombosis (P=0.027), non-response to first-line treatment (P=0.010), smoking (P=0.011), arterial hypertension (P=0.005), and obesity (P=0.041) revealed to be significant. The multivariate analysis model showed that older age at diagnosis (RR, 2.272; 95% CI, 1.167-4.426; P=0.016), ≥2 treatments (RR, 2.539; 95% CI, 1.305-4.941; P=0.006), persistent or chronic ITP versus acute (P=0.009), ≥2 treatment lines (RR, 2.539; 95% CI, 1.305-4.941; P=0.006), persistent or chronic ITP (versus acute, P=0.009), arterial hypertension (P=0.005), cigarette smokers were more likely to develop TEE. The knowledge about the risk of thromboembolic events in adult ITP patients could have an impact on management attitude for patients at risk.

Summary/Conclusions: Adult ITP patients are at risk for thromboembolic events. Patients older than 50 years, having a persistent/chronic form of the disease, requiring two or more lines to treat the ITP, previous splenectomy, and smokers were more likely to develop TEE. The knowledge about the risk of thromboembolic events in adult ITP patients could have an impact on management attitude for patients at risk.

Summary/Conclusions: Chronic ITP patient with anti-GPIbα autoantibodies who do not respond to conventional therapies and exhibit significant platelet desialylation may achieve a complete response to treatment with oseltamivir.

References

Quality of life, palliative care, ethics and health economics

E1457

BORT佐MIB THERAPY IS ASSOCIATED WITH SIGNIFICANT RESOURCE IMPLICATIONS FOR BOTH PATIENTS AND PROVIDERS: RESULTS OF A TIME-IN-MOTION STUDY

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Background: Bortezomib is a proteasome-inhibitor, which has improved outcomes in multiple myeloma (MM). Its use is approved within the UK NHS. Bortezomib is frequently administered as a subcutaneous injection in a hospital day treatment unit. Whilst the administration of a subcutaneous injection is brief, the process for the patient travelling to hospital, assessment and waiting for the delivery of the injection can take considerable time. From a patient perspective, significant amount of time spent without economic activity and travel costs add up during the course of therapy. From the health-care provider the process of safely administering bortezomib has significant resource implications beyond those of drug procurement.

Aims: We set up a time-in-motion study to evaluate the costs to health care provider and patients during bortezomib therapy to estimate the ‘real-world’ cost of delivering bortezomib therapy.

Methods: Retrospective data collection was undertaken, using electronic prescribing records for patients treated between July 2014 - August 2016. Travel distance and time was estimated using Google maps and costed using HMRC mileage (an approved costing of mileage used for taxation purposes). The NHS schedule of service costs was used to estimate the cost of bortezomib administration. Cost of delivery of Bortezomib for healthcare providers is a sum of these individual costs.

Results: We identified 127 patients who incurred a total of 2,134 visits whilst receiving Bortezomib therapy at the Churchill Hospital in Oxford during this 2 year period. Median age was 70 years-old (yo) (39-95); Male 74 patients (58%) 53 patients (42%). We restricted the analysis to 110 patients who started and completed therapy during the study period. Median number of patient visits was 16 (range 11-52). The median travel distance (return journey) for each patient was 33 miles (53 km) (range: 1.2-224 mi; 1.9-360 km). Median travel time was 90 min (range: 8-300 min). The range travel cost per patient was £8.35-£13.20. Twenty-seven patients (21%) required use of specialist hospital transport services, which resulted in 295 transport-episodes (14%) in total. In order to assess the time spent in the day therapy unit, a subgroup of 589 patient-episodes were analysed to assess time from arrival to administration of Bortezomib: the median time from patient registration to bortezomib administration was 63min (range: 5-433min). Pharmacy cost for preparation of Bortezomib was £50 per dose. The cost of delivery of bortezomib (not including cost of drug) was £1,160 per cycle, which equated to a total median cost of £4,640 per patient (range: £290-£15,080). Drug procurement costs for Bortezomib is estimated at an additional £12,261 per course of therapy (BNF 2016). Delivery costs therefore added an additional 38% to the procurement costs.

Summary/Conclusions: We provide the first time-in-motion data on myeloma patients treated with Bortezomib. The ‘real-world cost’ of delivering therapy is 37% higher than the drug-costs alone. In addition the impact on patients is substantial: over a two year period 127 patients required 2,134 visits with a median time in the day unit of 63 minutes and a median travel time of 90 minutes per visit. Our data highlights the burden of both time and economic costs to patients during therapy. Novel oral proteasome inhibitors offer the potential to reduce this resource impact in the future. This data could be used by health care providers and reimbursing agents for economic modeling of the potential benefits of oral proteasome inhibitors.

E1458

HOSPITAL CARE AT HOME ADMINISTRATION OF SUBCUTANEOUS AZACITIDINE IS FEASIBLE AND PREFERRED BY PATIENTS COMPARED TO HOSPITAL ADMINISTRATION: A FRENCH REGIONAL HEMATOLOGY NETWORK EXPERIENCE

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Background: In France, azacitidine (AZA) is indicated for the treatment of adult patients affected by Myelodysplastic Syndrome with intermediate-2 or high risk according to the International Prognostic Scoring System (IPSS). Chronic Myelomonocytic Leukemia (CML) with 10-29% medullary blasts and Acute Myeloblastic Leukemia (AML) with 20-30% blasts. It’s also a drug treatment of adult AML patients over 65 years with>30% of medullary blasts. Azacitidine is a hypomethylating agent administered orally. 4 weeks of effective, treatment cycles require frequent hospital visits which could decrease patient comfort and increase medical personnel workload. Limousin is a region with the oldest population of France and with a very low population density. There is one university hospital and two local state-run hospitals each with a hematology department. In 2009, HEMATOLOGICAL MALIGNANCIES: A RANDOMIZED CONTROLLED the oldest population of France and with a very low population density.

Methods:

Aims: Our work aimed to demonstrate that HaH administration of AZA is feasible and well preferred by patients compared to hospital administration. Patients were randomly assigned into two arms for receiving either subcutaneous AZA placebo plus LIA (standard group, 48.6%) or oral fentanyl citrate 200 mcg plus oral midazolam 5mg in addition to LIA (combo-group, 51.4%) during BMAB. Pre-procedural anxiety and procedural pain were assessed according to the Numered Rating Scale (NRS: 0-10), dividing the time of the procedure into five intervals (T0, T1, T2a, T2b, and T3) and evaluating discomfort grade during each moment of procedure in both groups. Cognitive function was measured before and 30 minutes after the procedure. Possible side effects were recorded, as well as the adequacy of tissue samples harvested. A telephone interview was performed 24 hours later. A total number of one-hundred-sixteen (n=116, Table 1) were enrolled in the study. Nine (n=9) patients did not meet inclusion criteria and were excluded. Fifty-two (n=52) patients were randomly assigned to standard group and fifty-five (n=55) to combo group.

Results: At T2b (corresponding to the biopsy time and time after the biopsy, respectively) there was a significantly lower (< 0.05) perception of pain in the patients who received sedo-analgesia (combo-group) compared to those who did not (standard group). Moreover, 100% of the patients in combo group who had previously undergone this procedure without premedication, reported that they would prefer sedoanalgesia for the subsequent procedures, thus showing the effectiveness of this combination also in relieving anticipatory anxiety. Finally, the histological specimen was found to be high in quality, as defined by standards.

Table 1.

E460

ASSESSMENT OF THE ECONOMIC IMPACT OF HORSE-ATG IN SWEDEN FOR APLASTIC ANAEMIA


Background: Aplastic anaemia (AA) is a rare, potentially fatal haematopoietic stem-cell disorder that can either be inherited or acquired. AA is graded according to disease severity, from non-severe to very severe and is linked to immune-related responses such as the destruction of bone marrow. Cases of severe and very severe AA are considered to be a haematological emergency requiring urgent treatment. Extended hospitalisations and the cost of treatments and disease management are associated with the economic impact of AA.

Summary/Conclusions: Administration of oral analgesia and anxiolysis is a safe and feasible option to be used in outpatient setting; sedo-analgesia is very effective in reducing pain during the biopsy and diminishes the anticipatory anxiety related to a painful procedure. Patients should have the possibility to choose between local anaesthesia alone or sedo-analgesia plus local anaesthesia.
Aims: To assess cost-effectiveness of ATGAM (horse antithymocyte globulin) in comparison to rabbit antithymocyte globulin (r-ATG) in the treatment of moderate to severe aplastic anaemia (sAA) patients in Sweden.

Methods: A semi-Markov state-transition cohort model was developed to estimate long-term (up to 5 years) clinical and economic outcomes for patients with AA receiving either ATGAM or r-ATG as first-line IST treatment. The following key assumptions were included in the model: responders who relapse are assumed to be re-treated with no expected change in survival. Patients who do not respond to first-line treatment move onto a second-line treatment comprised of either IST, IST + etrombopag or hematopoietic stem cell transplantation (HSCT). Although response rates are lower, those who respond to second-line treatment are assumed to have the same outcomes as those who respond to first-line. Patients who continue to not respond receive standard supportive care with a significant decrease in expected survival. Efficacy data for ATGAM and r-ATG were obtained from published literature. Adverse events were not included due to lack of evidence of any difference between the two comparators. Medication, administration, and disease management costs were obtained from published literature, publicly available sources and clinical expert opinion. As resource utilization for disease management changes over time and differs considerably between responders and non-responders, three distinct phases have been included in the model: short-term (first 6 months post-IST administration), medium-term (6-12 months) and long-term (greater than 1 year), for patients in either of the response categories.

Results: Response to treatment was calculated to be seen in 67% of ATGAM patients' vs 35% in r-ATG (accounting for mortality). Over 5 years, the model estimated that patients gained 4.15 life-years (3.28 quality-adjusted) on ATGAM vs 3.52 (2.56) on r-ATG. Short-term disease management costs were estimated to be SEK 880,144 (€98,816) in responders vs SEK 1,264,016 (€139,041) in non-responders. Medium and long-term costs also followed the same pattern. Overall costs (drug plus disease management), were significantly lower for patients receiving ATGAM vs r-ATG; making ATGAM cost-saving by being both more effective and less costly than r-ATG. When considering treatment costs only (including cyclosporine and HSCT), the model estimated a cost of SEK 107,097/life-year gained (€11,781) and SEK 135,655/quality-adjusted life-year (€14,192), showing ATGAM is highly cost-effective. The analysis showed that when treatment and disease management costs are considered, ATGAM dominates r-ATG as the gain in QALYs and LYs are achieved at a lower cost. Therefore making ATGAM cost-saving with greater health benefits in comparison to r-ATG.

Summary/Conclusions: Due to improved treatment response, survival, and quality of life outcomes, the model shows that ATGAM is at least more cost-effective, if not cost-saving, in comparison to r-ATG for the treatment of patients with aplastic anemia.

A CLINICAL AUDIT OF NUTRITIONAL SCREENING AND SUPPORT OF HOSPITALIZED PATIENTS WITH HEMATOLOGIC DISEASES

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Background: Poor food intake is a common problem in patients with hematologic diseases. Recurrent infections and chemotherapy complications are some of the possible causes. Malnutrition is correlated to slow recovery, prolonged hospitalization, and higher mortality. Audits about the nutritional support of hospitalized patients may detect significant failures in patient care and help towards the correct application of the international guidelines.

Aims: We performed a prospective observational audit on hospitalized patients with hematologic diseases to investigate their nutritional status and whether they received the appropriate nutritional support.

Methods: The initial population consisted of 122 consecutive patients with hematologic diseases admitted from March 31, 2016 to June 8, 2016 in two Hematologic Units of a Tertiary University Hospital in Athens, Greece. We designed a special questionnaire based on the Malnutrition Universal Screening Tool (MUST) with additional questions on demographic, somatometric and medical data (Table 1). The questionnaire was applied by 6th-year medical students to all patients within 48 hours of admission. Patients were classified as high, intermediate, and low-risk per the MUST score and were reassessed at predefined intervals. During reassessment, we examined the food intake and the nutritional interventions (nutritional supplements, enteral or parenteral nutrition) applied.

Results: Ninety-three patients were included in the final analysis (5 refused to participate, 22 were excluded due to short-term hospitalization, 2 were absent during reassessment). Forty-one (38%) patients had a MUST score ≥2 (high risk) but none of them received nutritional supplements. One patient was supplemented with parenteral nutrition (Table 1).

<table>
<thead>
<tr>
<th>Table 1: Patients’ characteristics and results</th>
</tr>
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<tbody>
<tr>
<td>Number of patients, N (%)</td>
</tr>
<tr>
<td>Age (years), median (range)</td>
</tr>
<tr>
<td>Gender (M/F)</td>
</tr>
<tr>
<td>BMI (kg/m²), median</td>
</tr>
<tr>
<td>% of unplanned weight loss in past 6 months, median (range)</td>
</tr>
<tr>
<td>Disease, N (%)</td>
</tr>
<tr>
<td>Leukemia</td>
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<tr>
<td>Lymphoproliferative disorders / Multiple myeloma</td>
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<tr>
<td>Acute leukemia / Myeloproliferative disorders</td>
</tr>
<tr>
<td>Benign hematologic disorders</td>
</tr>
<tr>
<td>% of non-confronted diagnosis</td>
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<tr>
<td>MUST, N (%)</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
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<tr>
<td>2</td>
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<tr>
<td>3</td>
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<tr>
<td>% of patients receiving nutritional support, N (%)</td>
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<tr>
<td>Recorded food intake (last 5 days), N (%)</td>
</tr>
<tr>
<td>Increased</td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Decreased</td>
</tr>
<tr>
<td>Decreased by more than 25%</td>
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<tr>
<td>Serum albumin levels on admission/discharge, g/dl (median, range)</td>
</tr>
<tr>
<td>Other variables: estimated food intake in 3 days, reduced appetite, type of diet, calorie intake, duration of hospitalization, ECOG score, recent surgery, dysphagia, nausea, mucositis, infection, neurological deficits, head trauma etc.</td>
</tr>
<tr>
<td>*65 year old woman with 12% weight loss over the last month, BMI≤18.5 kg/m², alb&lt;2g/dl, a hospital-acquired infection and no food intake.</td>
</tr>
</tbody>
</table>

Summary/Conclusions: Our audit revealed a lack of nutritional support of the hospitalized patients. A meeting with the involved health professionals was organized and an oral presentation of the results and the possible causes (lack of sensitization of the staff, high regimen cost, shortness of staff) was performed. Proposals to change the current situation were made such as detection of high risk patients by medical students and further assessment by a nutritional specialist. A brief MUST-based questionnaire was also proposed to be used for all patients upon admission. A re-audit was programmed and is already in progress.

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E1463
ASSESSING REAL-WORLD TREATMENT PATTERNS, OUTCOMES AND RESOURCE USE IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) POST AUTOLOGOUS STEM CELL TRANSPLANT ACROSS EUROPE
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Background: Autologous stem cell transplant (ASCT) is the standard of care for first line (1L) treatment (tx) for patients (pts) with MM deemed of suitable fitness to safely undergo the procedure. More recently introduced tx options have significantly increased the life expectancy of pts with MM and continue to provide further promise for the future in this devastating disease. The increasing therapeutic armoury across the MM pathway allows for varied tx patterns providing both potential differences in outcomes and healthcare resource use (HCRU).

Aims: The aim of the analysis was to determine current management of pts in the post ASCT setting, assess outcomes of pts and HCRU.

Methods: A retrospective chart review was conducted in France, Germany, Italy, Spain and the UK. Data collection took place in Q1 2017. Physicians provided data on consecutive pts with MM who had undergone an ASCT as part of 1L tx or on after 1st January 2014, to specifically examine the HCRU post 1L SCT. Data collected pertained to pt characteristics, b tx patterns, duration of tx and outcomes (including time to progression (TTP) and best response achieved (IMWG updated criteria), HCRU in terms of hospitalizations, additional supportive drugs prescribed and healthcare professional (HCP) visits. Pt records included in this interim analysis were completed by Feb 17th 2017, with data collection continuing in all countries.

Results: 214 record forms have been reviewed to date. Pts’ mean age at diagnosis was 59 (±7.8 SD) years; 43% female and 57% male. Mean duration from diagnosis, to receiving an ASCT was 9.6 months (±13.3 SD). Of the pts included in the study, 62%, 28% and 8% had received 1st, 2nd and 3rd line tx respectively. In the 1L setting, 72% of pts did not receive any drug therapy post ASCT, 21% received consolidation and 5% maintenance therapy. Of the pts who did not receive maintenance therapy, 42% and 34% went onto receive 2L and 3L drug therapy respectively; whereas, only 24% of pts who received maintenance therapy went onto 2L, and none onto 3L. The most frequently prescribed regimens at 1L maintenance were Lenalidomide (82%), Bortezomib (12%) and Thalidomide (9%). The TTP from start of tx was 22.2 months (±11.1 SD) for pts not receiving maintenance and 33.0 months (±8.1 SD) for pts receiving maintenance. Overall 43% of pts achieved a sCR and CR, 51% achieved a VGPR and PR. During the period from 1L post ASCT to start of 2L, 54% of pts required supportive tx (bisphosphonate (55%), blood transfusions (24%), G-CSF (21%), ESAs (11%), radiotherapy (4%) and dialysis (3%)). 64% of pts were hospitalized at least once during this period, with a mean duration of 7.2 days (±18.1 SD). The mean number of visits to Hematologists was 7.1 times in 24.8 months (between start of 1L to start of 2L b); mean visits to a HCP during this period were 17. The mean TTP from start of 2L tx was 11.2 months (±6.0 SD); 20% of pts achieved a sCR and CR, 52% achieved a VGPR and PR.

Summary/Conclusions: The sample is reflective of the pt demographics data reported in Raab et al. 2016. Furthermore, the TTP for pts not receiving any active ongoing tx post ASCT in this real-world study is comparable to findings in the literature. There is no data exists on HCRU post ASCT. This study demonstrates that there is ongoing HCRU impact even if pts are not receiving any active ongoing tx post first ASCT. Prolonging the remission period post ASCT may therefore spread the marginal cost of HCRU whilst simultaneously enhancing a pt’s quality of life by deferring future tx lines.

E1464
NUMBER-NEEDED-TO-TREAT (NNT) AND COST OF RESPONSES ACHIEVED IN TYROSINE KINASE INHIBITOR (TKI) TREATMENT OF REFRACTORY CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA (CP-CML) IN THE UNITED STATES (US)
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Background: The emergence of targeted therapies with high efficacy in small patient populations such as TKI-refractory CP-CML has challenged decision-makers.

Aims: To demonstrate a simple and intuitive approach to assessing the value of available TKIs (nilotinib, dasatinib, ponatinib and bosutinib) in this setting.

Methods: Using synthesized efficacy data from a published meta-analysis (Liu et al. 2014) and cost data from current practice, we calculated NNT to achieve one additional response—i.e., the multiple of treated patients to responders. We assumed response is not evaluated prior to 3 months, per National Comprehensive Cancer Network (NCCN) guidelines. Therefore, the cost of achieving an additional response was estimated as the product of NNT and 3-month cost, based on US Wholesale Acquisition Costs (WAC) and recommended dosing for each TKI from US prescribing information (USPI).

Results: To achieve one expected response, the NNT is 1.7 (95%CrI: 1.5-1.9) patients for ponatinib, 3.8 (3.4-14.8) for nilotinib, 4.2 (2.2-11.1) for dasatinib, and 4.5 (3.4-6.7) for bosutinib (based on CCyR of 60%, 26%, 24% and 22%, respectively). With a 3-month WAC for ponatinib of $49,883, nilotinib: $33,892, dasatinib: $33,897 and bosutinib: $36,045, the estimated 3-month cost per response achieved is $82,000 ($73,100-$95,500) for ponatinib, $130,000 ($108,000-$161,000) for nilotinib, $141,000 ($75,300-$377,000) for dasatinib, and $164,000 ($124,000-$240,000) for bosutinib.

Summary/Conclusions: Using published, synthesized efficacy estimates, the NNT to achieve one response with ponatinib in TKI-refractory CP-CML is less than with nilotinib and dasatinib, whereas it is comparable with bosutinib. The lowest estimated 3-month cost per response achieved. Therapy choice should, however, consider both treatment cost and the benefit-risk profile of the individual patient.

E1465
THE COST-EFFECTIVENESS OF PEGASPARAGASE FOR FIRST-LINE TREATMENT OF ACUTE LYMPHOBLASTIC LEUKAEMIA: A COST-UTILITY ANALYSIS
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Background: Asparaginase is a key component in the multi-agent chemotherapeutic regimen for the treatment of children, adolescents, and adults with acute lymphoblastic leukaemia (ALL). Compared to native asparaginase (native ASP), pegaspargase (PEG-ASP) has a longer half-life, can be given less frequently, and is less immunogenic, which leads to fewer hypersensitivity reactions. In the UK, patients with newly diagnosed ALL are treated with PEG-ASP followed by Erwinia-derived asparaginase (ERW-ASP) in cases of hypersensitivity, based on the UKALL protocols. Although native ASP is no longer used as the first choice of asparaginase therapy, it was the standard of care before PEG-ASP was available. A cost-utility analysis (CUA) was conducted to evaluate overall cost-effectiveness of PEG-ASP in comparison to native ASP when utilized as part of antineoplastic combination therapy for treating newly diagnosed ALL in children, young people, and adults.

Aims: To evaluate the cost-effectiveness of a treatment strategy including PEG-ASP in a multi-agent in patients with newly diagnosed ALL compared to regimens that include native ASP.

Table 1.
**Results:** The base-case scenario demonstrated that PEG-ASP followed by ERW-ASP dominated (i.e., was both less costly and more effective than) native ASP followed by ERW-ASP in adults, children, and the whole (combined) population (Table). Scenario analyses highlighted the robustness of the cost-effectiveness results. Differences in total QALYs between PEG-ASP and native ASP were driven primarily by the difference in hypersensitivity rates.

**Summary/Conclusions:** This analysis demonstrates that PEG-ASP, as part of multi-drug chemotherapy, is a cost-effective treatment option compared to native ASP for treating ALL in children, young people and adults with newly diagnosed ALL.

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**E1466**

**IMPACT OF VENETOCLAX ON THE QUALITY OF LIFE OF PATIENTS WITH RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA: RESULTS OF A PHASE 2, OPEN-LABEL STUDY OF VENETOCLAX (ABT-199/ GDC-0199) MONOTHERAPY**

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**Background:** Chronic lymphocytic leukemia (CLL) is associated with reduced health-related quality of life (HRQoL), with progressive severe fatigue being a particularly relevant burden. Disease-related symptoms, toxic effects of therapy, and the awareness of living with an incurable disease can have a profound impact on HRQoL. Venetoclax (VEN) is an oral BCL-2 inhibitor that has demonstrated deep and durable responses in relapsed/refractory (R/R) CLL patients.

**Aims:** To assess whether Venetoclax has a sustained impact on health related quality of life among patients with relapsed/refractory CLL based on a second interim analysis (first interim results through week 24) of patients treated with VEN monotherapy.

**Methods:** Patients ≥18 years of age with R/R CLL received VEN monotherapy until disease progression, unacceptable side effects, or discontinuation for any other reason. Patient-reported HRQoL measures included the EORTC QLQ-C30 and EORTC QLQ-CLL16 global health status and the role, social, and emotional functioning scales. Improvements in VEN treated patients in EORTC-QLQ-CLL16 disease effects, social problems, and future health worries scores were statistically significant and exceeded the MID at all assessment points. Furthermore, early and sustained improvements in fatigue through week 96 were seen in both EORTC-QLQ-C30 and EORTC-QLQ-CLL16 (Table 1). The changes observed in patient EORTC-QLQ-CLL16 future health views were considered large (>20 points) at all assessment points. Clinical relevance was based on minimum important difference (MID) of values from BL at different assessment points. Differences in total QALYs between PEG-ASP and native ASP followed by ERW-ASP in adults, children, and the whole (combined) population were driven primarily by the difference in hypersensitivity rates.

**Summary/Conclusions:** This group of medical students found it difficult to correctly diagnose some of the haematological conditions presented, even though they had studied all the conditions before, however the use of a ‘Team Based Learning’ approach where students could discuss the cases in small groups did improve their results. Interestingly for two conditions, for CML and Multiple myeloma the number of correct answers was the same for i-RAT and t-RAT, possibly the students who responded correctly during the i-RAT were concentrated in fewer groups. To the author’s knowledge this is the first study on the effect of applying the Team Based Learning method to haematology teaching. This study showed that TBL could be a useful teaching tool to improve teaching of haematological conditions in medical schools, however the size of the sample was small and the results should be validated with a bigger study.

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**E1467**

**WHICH HAEMATOLOGICAL CONDITIONS CAN THIRD YEAR MEDICAL STUDENTS RECOGNIZE INTERPRETING FULL BLOOD COUNT RESULTS?**

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**Background:** Haematology is often seen by medical students as a niche specialty, however interpreting full blood count results is a daily job for most hospital doctors. Furthermore a recent review showed that survival for haematological malignancies is worse in UK than in other European countries due to late diagnosis being one the possible causes. Educate future doctors in interpreting symptoms and blood results correctly to suspect haematological condition should be considered essential. In the UK the Education Subcommittee of British Society for Haematology wrote the ‘A Haematology Curriculum for Medical Students’ as a guide to the knowledge of haematology expected from medical students.

**Aims:** The aim of the study was to evaluate the ability of a group of third year medical students in recognising ten haematological conditions as indicated in the curriculum proposed by the BSH. The students had all attended a haematology course during their second year of medical school.

**Methods:** A multiple choice test “best of four” containing ten clinical cases including full blood count results was given to the students. According to the Team Based Learning “TBL” model the students completed the test first individually, ‘i-RAT’ and then after discussing the results in small groups ‘t-RAT’. The topics and the percentage of correct answers are shown in Table 1.

**Results:** Twenty four students participated. In the i-RAT none of the scenarios were correctly interpreted by 100% of the students, the scenarios interpreted correctly by at least 70% of the students were only two: B12/folate deficiency and iron deficiency; less than 30% of the students could identify CML, NHL and Multiple myeloma; the remaining topics: thalassemic trait, MDS AML, CLL and blood results correctly to suspect haematological condition should be considered large (>20 points) at all assessment points. Clinical relevance was based on minimum important difference (MID) of values from BL at different assessment points. Differences in total QALYs between PEG-ASP and native ASP followed by ERW-ASP in adults, children, and the whole (combined) population were driven primarily by the difference in hypersensitivity rates.
Methods: A non-interventional, longitudinal online study was conducted among patients with AL amyloidosis who were recruited with assistance from patient advocacy groups. Initial (n=341) and six-month follow-up (n=226) surveys assessed demographics, disease and treatment characteristics, and health-related quality of life (HRQoL), measured by the SF-36v2® Health Survey physical and mental component summary scores (PCS and MCS). HCU (e.g., outpatient visits, hospitalizations, and treatment) was measured during the six-month follow-up. Prevalence of HCU and its bivariate associations with patient characteristics were evaluated. Multivariable logistic regression models were used to test for associations between HRQoL and having an ER visit or hospitalization in the past six months.

Results: Overall, visits with specialists and other healthcare providers during the previous six months were nearly ubiquitous (92.0% and 94.6%, respectively). Collectively, 56.0% of patients reported having ≥1 ER visit or hospitalization. ER visits and hospitalizations were not associated with the numbers or types of organ systems affected by the disease or the duration of disease. There were significant associations between PCS and ER visits (p<0.05) and between both PCS and MCS and hospitalizations (p<0.05 for all) based on multivariable analyses.

Summary/Conclusions: There is a lack of real-world evidence regarding HCU among patients with AL amyloidosis. This research identified longitudinal associations between HRQoL and HCU, indicating there is potential for using HRQoL surveys as screening tools to predict future HCU for AL amyloidosis patients. The development of prediction models for HCU in AL amyloidosis should consider incorporating HRQoL, as well as disease staging and treatment type.

E1469

SAFETY, FEASIBILITY AND EFFECTIVENESS OF ELECTRICAL MUSCLE STIMULATION IN HOSPITALIZED PATIENTS UNDERGOING AUTOLOGOUS OR ALLOGENEIC STEM CELL TRANSPLANTATION AND INTENSIVE CHEMOTHERAPY

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Background: Autologous and allogeneic stem cell transplantation (HSCT) or intensive chemotherapy are the only treatment option for many patients with haematological malignancies. Even after complete remission many patients are physically and psychologically impaired because of intensive treatment and weeks of immobilisation. Electrical muscle stimulation (EMS) is a verified training tool to prevent muscle decline in seniors and helps improving physical performance in patients with chronic diseases.

Aims: This prospective, randomized and controlled study tested the safety, efficacy and fidelity of EMS in 72 patients (EMS=42, control=30) undergoing autologous HSCT (n=21), allogeneic HSCT (n=17) and intensive chemotherapy (n=34).

Methods: A Myopuls 2000 device (Curatec Services Gmbh) was used. Targeting training time was 15 minutes 5 days a week on both thighs and arms from start of therapy (T1) to time of discharge (T2). Adverse events and treatment adherence were documented. Impact on psychological and physical functioning was evaluated using the Multidimensional Fatigue Inventory (MFI), the EQ5D QLC-C30, the Short Physical Performance Battery and the 6 Minute Walking Distance at T1 and T2.

Results: Seven patients died in the EMS- (n=4) and control-group (n=3). 6 of 42 EMS patients withdrew because of sepsis (n=4) or loss of motivation (n=2). 32 patients from the EMS group completed our study with 22 accomplish- ing ≥60% of the pre-set training time. EMS related adverse events were intramuscular hematoma (n=1) and muscle pain (n=2). No bleeding events (WHO bleeding scale=1) or ventricular arrhythmies occurred. Difference in 6-minute walking distance between both groups was 23 meter (p=0.2). SPPB test results differed by one point (p=0.08). MFI and EQ5D QLC-C30 both favoured the EMS group, but showed no statistical significance.

Summary/Conclusions: EMS is feasible and safe in patients undergoing intensive chemotherapy regimens. It also may improve physical fitness, fatigue and quality of life, indicated by favourable test results in the EMS group. To verify positive effects of EMS in patients with haematological malignancies, further research is needed, with more patients and sham EMS stimulation.

E1470

MYELOMA PATIENT VALUE MAPPING: A DISCRETE CHOICE EXPERIMENT

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Background: Myeloma is a life threatening haematological cancer. Although myeloma is responsive to treatments, there remains no cure. In recent years, there have been improvements in survival due to the use of high dose therapies, stem cell transplant, and other novel therapies. However, while myeloma patients are living longer, they are also living with symptoms and treatment related toxicities. Therefore, myeloma patients face difficult decisions about the benefits and risks of treatment. The purpose of this study was to assess myeloma patient preferences for treatment.

Aims: The study aimed to answer the following questions: What treatment attributes do myeloma patients value? What is the relative importance of different treatment attributes to myeloma patients and the maximum acceptable risk they are willing to accept? What risk-benefit trade-offs characterise patients’ decision-making around treatment options, including not to treat? What, if any, influences and predictive factors are found in the way patients assess benefits and risk?

Methods: Participants were 475 Myeloma patients in the UK. Data were collected using discrete choice experiments (DCEs) through an online survey. The DCEs presented patients with a traditional treatment choice experiment (e.g., treatment A vs treatment B), focusing on the clinical benefits of treatments and the associated risks. The attributes and levels of the attributes were selected based on previous research, literature review and expert opinion. The DCE data were modelled using a Latent Class Model (LCM), and the effect of demographic characteristics were also examined.

Results: Findings revealed two classes (groups) of patients with different preferences for treatments. Patients in class one placed greater importance on overall survival and mild-to-moderate side effects, whereas patients in class two placed greater importance on how the treatment was administered and the treatment adherence. Results of the LCM model explained 49% of the variance.

Summary/Conclusions: Findings from this study suggest that not all myeloma patients value the same treatment features equally. This finding has important implications for further healthcare policy decision making and could be important for evaluating the impact of the use of subcutaneous formulations of rituximab.

E1471

COST-MINIMIZATION ANALYSIS OF RITUXIMAB SUBCUTANEOUS FORMULATION VERSUS INTRAVENOUS ADMINISTRATION OF RITUXIMAB FOR THE TREATMENT OF NON-HODGKIN’S LYMPHOMA IN THE REPUBLIC OF MACEDONIA

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Background: Rituximab, an anti-CD20 monoclonal antibody, in combination with chemotherapy is a standard of care for non-Hodgkin’s lymphoma (NHL), in which it is administered as a standard dose of 375mg/m2 body surface area (BSA), intravenously (IV) infusion, or fixed dose of 1400mg administered as subcutaneous formulation (rituximab SC). Intravenous infusion of rituximab typically last for three to four hours, while subcutaneous application last approximately five to seven minutes. The evidence to support the use of rituximab SC as an alternative to rituximab IV is primarily based on the phase III, randomised, non-inferiority, open-label SABRINA study. Recent studies demonstrated therapeutic and pharmacokinetic non-inferiority of rituximab SC to rituximab IV.

Aims: The aim of the study was to identify and compare the total costs of subcutaneous (SC) vs intravenous (IV) administration of rituximab for the treatment of NHL patients in the Republic of Macedonia.

Methods: Cost-minimization analysis was used to evaluate pharmacoeconomic impact of the use of subcutaneous vs intravenous administration of rituximab in the treatment of NHL patients. The total of 220 NHL patients (mean body surface area 1.9 m2, middle aged 59.6 years) were enrolled in the study. Evaluated healthcare resources included drug treatment costs, infusion chair occupying cost, active Healthcare Professional time cost and consumable disposals.

Results: Direct costs of administering one course of rituximab, including cost of drug, cost of administration and cost of consumables in all treatment phases (premedication, medication and post medication), for intravenous administration of rituximab were 162€ compared to 1546€ for subcutaneous administration of rituximab. Average time for intravenous administration is 6 hours, 12 minutes and 13 seconds, compared to 10 minutes and 13 seconds for subcutaneous administration. Subcutaneous rituximab incurred less non-drug related costs than intravenous rituximab under the observed clinical practice: 14.62€ vs 1.76€ regarding active healthcare professional time and 10.10€ vs 1.2€ as infusion chair occupying cost.

Summary/Conclusions: Subcutaneous administration of rituximab is a cost-saving therapy in comparison with intravenous administration of rituximab for the treatment of NHL patients in the Republic of Macedonia.
E1472
QUALITY OF LIFE AND ABILITY TO WORK OF PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA TREATED WITH THYROSINE KINASE INHIBITORS
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Background: Thyrosine kinase inhibitors (TKIs) are now standard treatment for chronic myelogenous leukaemia (CML), but little is known about quality of life (QoL) of the patients.

Aims: The purpose of this study is to evaluate QoL of CML patients receiving TKIs, a disease requiring strict daily compliance with taking these drugs orally, as well as regular clinical and biological controls.

Methods: The study included patients with CML followed in three hospitals in west Algeria between 2004 and 2016. The measure of QoL was performed by the tool of functional assessment of chronic illness therapy (Functional Assessment of Chronic Illness Therapy, FACIT) for leukaemia. We have established QoL scores given by the questionnaire, FACIT, consisting of three levels: TOI for leukaemia trial outcome index, FACT-G for general score, and FACT-LEU for the total score of leukaemia. Specific areas of the questionnaire were associated with QoL of patients such as fatigue and ability to work. The correlation between the three TOI, FACT-G and QoL scores was assessed using Spearman’s test. The test is significant if p<0.05.

Results: 67 patients with CML have agreed to answer to the questionnaire of QoL, medications in use, and their side effects. The mean QoL of the patients was 93.7 (out of 124 total points) for the TOI, 77.2 (out of 108) for the FACT-G, and 128.9 (out of 176) for the FACT-LEU. Patients who presented with TKIs side effects had a low score of QoL (p=0.0006), especially when these effects are severe (p=0.003). Stopping TKIs medication was noted in 41.3% of patients with severe side effects. Severe fatigue was observed in 14 (22.9%) patients, having low QoL scores in all scales (p<0.0001). 44 (65.8%) patients were able to work with higher QoL scores in the three FACIT scales (p<0.0001, Spearman correlation).

Summary/Conclusions: QoL is an important aspect in the management of CML, its assessment is necessary and must be regular. The ability to work and fatigue are important components of QoL of patients receiving TKIs and should be specifically taken into account during the treatment. Adverse effects of TKIs can interfere with QoL of patients and can lead to discontinuation of CML therapy.

E1473
QUALITY OF LIFE AND EMPLOYMENT AFTER AN HEMATOPOIETIC STEM CELL TRANSPLANTATION IN A MEXICAN POPULATION
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Background: Hematopoietic stem cell transplantation (HSCT) is a consolidation therapy for multiple hematological malignancies and its goal include patients achieve levels of quality of life (QOL) similar that general population. However, studies developed in Europe and United States have shown that patients on long-term follow-up after HSCT reported lower levels of QOL, more unemployment and lower household income than before the procedure. These relationships have not been examined in Mexican HSCT patients.

Aims: To describe the QOL (EORTC-QLQ), level of employment and household income in Mexican patients on follow-up after HSCT

Methods: This was a cross-sectional study with patients ≥18 years old with at least one year of follow up after HSCT at the National Cancer Institute, Mexico. Results: 30 participants were included, with a median age of 34 years (range 25-64), 56% male, and 41% married. Regarding educational level 68.7% had basic education, 25% had a college education and 6.3% postgraduate education. Mean time after HSCT was 36 months, 10% had active chronic graft versus host disease (GvHD). Patients reported moderate to high levels of QOL (Table 1). With respect to employment, 52% had a job (56% had a full time job, 13% work part-time and 31% had an informal job) and 48% were unemployed (50% could not find a job and 50% did not want to have a job). Finally, 56% had lower household income than before HSCT.

Summary/Conclusions: Mexican patients showed similar or higher levels of QOL in comparison with samples from other countries, with the exception of higher impact in emotional QOL and better social QL in our sample. Additionally, a substantial minority of patients were unemployed and over half had lower household income after HSCT. More work is needed to identify risks associated with changes in QOL, employment status and income among long-term survivors of HSCT.

E1474
ANTHRACYCLINE INCREASES THE RISK OF DEVELOPING DIABETES IN B CELL LYMPHOMA
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Background: Treatments of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) or R-CHOP like regimens have made B cell lymphoma to be one of the most curative hematological malignancies. Among the effective chemotherapeutic agents in B cell lymphoma treatment, anthracycline plays an important role. However, anthracycline associated bone marrow suppression and cardiotoxicity limit its clinical application. Whether anthracycline would further increase the risk of developing diabetes in B cell lymphoma remains unclear.

Aims: The aim of this study was to compare the cumulative incidences of diabetes in B cell lymphoma patients treated with and without anthracycline. We also investigated the dose effect of anthracycline on diabetes development. Additionally, whether anthracycline would increase the severity and complication of diabetes in B cell lymphoma patients were also studied.

Methods: We conducted this population-based study by using Taiwanese National Health Insurance Research Database. From 2004 to 2011, medical records from a total of 3894 B cell patients were analyzed. To understand whether anthracycline therapy was associated with more diabetes in B cell lymphoma, we compared the cumulative incidence of newly diagnosed diabetes between patients with (n=3147) and without (n=837) anthracycline treatments.

Results: Log-rank test did not show the difference of cumulative incidences of newly diagnosed diabetes between B cell lymphoma patients with and without anthracycline treatments (p=0.1448). However, anthracycline remained associated with more diabetes [hazard ratio (HR): 1.59; 95% confidence interval (CI): 1.05–2.39; p=0.0278] after adjustment for age, gender, and comorbidities. Moreover, cumulative anthracycline doses of 253–400mg (HR: 1.94; 95% CI: 1.23–3.05; p=0.0043) and 401-504mg (HR: 1.83; 95% CI: 1.11–3.01; p=0.0180) increased the incidence density of diabetes in a dose-dependent manner (p=0.0438). Notably, patients with and without anthracycline treatment had similar yearly adapted diabetes complications severity index alteration (0.58±1.89 vs 0.75±1.85; mean±standard deviation), suggesting anthracycline did not deteriorate outcomes of diabetes in B cell lymphoma patients (p=0.4924).
Summary/Conclusions: Anthracycline therapy was responsible for more diabetes in B cell lymphoma in a dose-dependent manner. More intensive blood sugar monitoring and control should be recommended to B cell lymphoma patients, especially those who received anthracycline treatment.

E1475
THE COST-EFFECTIVENESS OF LENALIDOMIDE PLUS DEXAMETHASONE FOR THE TREATMENT OF RELAPSED OR REFRACTORY MULTIPLE MYELOMA IN CHINA
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Background: The introduction of lenalidomide plus dexamethasone (RD), and bortezomib-containing regimens, has improved the management of relapsed or refractory multiple myeloma (rMM) in China. However due to the absence of both head-to-head (direct) comparative efficacy and local economic data, stakeholders still face hard choices to make when choosing one therapy over another. Indirect treatment comparisons and health economic modeling can help support decision-making by enabling the incorporation of country-specific unit cost data, in the comparison of cost-effectiveness of one treatment vs another where treatments have not been directly compared in clinical trials.

Aims: To assess the cost-effectiveness of RD relative to bortezomib/dexamethasone (VD) and bortezomib/cyclophosphamide/dexamethasone (VCD) for rMM in Chinese patients.

Methods: The Markov-based decision analytic model was constructed to simulate lifetime health benefits and direct medical costs associated with RD, VD, and VCD for rMM in Chinese patients. A systematic literature review was conducted (in both Chinese and English databases, from 2005 to 2016) to obtain efficacy data of the three treatment regimens. The risk of progressive disease associated with RD and VD were estimated from available Chinese trials. The efficacy of VCD and the mortality associated with progressive disease after treatments with RD and VD were lacking in China, therefore were estimated from the published international randomized clinical trials. Published quality of life data was adapted to Chinese rMM patients with health utility adjustment. The model took into account (i) drug acquisition costs, (ii) treatment administration (inpatient and outpatient) costs associated with Chinese urban setting, (iii) serious adverse events management costs based on a survey of seven MM centers across China, and (iv) rMM management costs estimated from a Chinese real-world hospital setting. Quality-adjusted life years (QALY) and direct medical costs in China, and (v) rMM management costs estimated from a Chinese real-world hospital setting.

Results: Based on the model simulation without discounting survival outcomes over a lifetime horizon, RD could obtain longer average PFS years than VD (1.41) and more discounted lifetime medical costs (¥149,706) and VCD (¥150,774) were less than the cost-effectiveness threshold of China (three times of estimated 2016 China GDP per capital ¥166,920/QALY, ¥1= €0.138). The cost-effectiveness of RD was compared with VD and VCD, respectively from the Chinese healthcare payer’s perspective. One-way sensitivity analysis and probabilistic sensitivity (PSA) with 5,000 Monte Carlo simulations assessed the impact of the model uncertainty on the cost-effectiveness of RD. A scenario analysis was conducted by meta-analysing the published international randomized trials for the efficacy associated with RD, VD, and VCD, to verify the base case analysis.

Summary/Conclusions: The findings of the content validation, pretesting and cognitive interviews indicate that HM-PRO possesses a strong content validity in different HMs, includes all the issues important to these patients and the statements are easy to read, understand and respond to spontaneously. HM-PRO will undergo further psychometric testing to support its psychometric properties across different types of HMs.

E1477
OVARIAN TISSUE CRYOPRESERVATION IN PEDIATRIC AND ADOLESCENT PATIENTS UNDERGOING CANCER CHEMOTHERAPY AND/OR HEMATOPOIETIC STEM CELL TRANSPLANTATION
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Background: Ovarian tissue cryopreservation (OTC) and subsequent reimplantation is the only option available for fertility preservation in prepubertal females, but this approach remains unestablished in pediatric and adolescent patients with cancer. After the experience of OTC for more than 200 patients with primary ovarian failure and more than 50 patients with breast cancer in our center over 5 years, we have started OTC for pediatric and adolescent cancer patients since 2015.

Aims: To define safety and benefits of OTC in pediatric and adolescent patients with undergoing cancer chemotherapy and/or hematopoietic stem cell transplantation.

Methods: From December of 2015 to February of 2017, OTC was performed in 6 girls (median age 14 years, range 11-15): 2 patients with myelodysplastic syndrome, 2 with lymphoma, 1 with acute lymphoblastic leukemia, and 1 with acute myeloid leukemia. Indications for OTC were 5 hematopoietic stem cell transplantation and 1 sterilizing chemotherapy. Two patients with myelodysplastic syndrome and 1 with immunodeficiency received no previous chemotherapy and the other 3 had received prior chemotherapy. Laparoscopy was used to collect a pair of ovaries that was frozen by vitrification method. Frozen ovarian tissue was cryopreserved in a 6% DMSO solution and all procedures were performed without major postoperative complications and this procedure did not delay chemotherapy or hematopoietic stem cell transplantation. Histological analysis of ovarian tissue revealed primordial follicles, even in the patients with previous cancer chemotherapy. No malignant cells were identified. Median post-harvest...
follow-up was 9 months (0-14) and all patients were alive. Hormonal results were evaluated for 3 patients; 2 patients were in premature ovarian insufficiency. Re-implantation of ovarian tissue has not yet been performed.

**Summary/Conclusions:** Although OTC and subsequent re-implantation is experimental, this approach may be the best method for restoration of ovarian function and fertility preservation in pediatric and adolescent cancer patients. A risk of reseeding malignant cells is a problem still to be conquered.

**E1478**

**A MULTI-DISCIPLINARY APPROACH TO CHEMOTHERAPY PRESCRIBING AT NEWCASTLE UPON TYNE HOSPITALS NHS FOUNDATION TRUST**

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**Background:** Newcastle Upon Tyne Haematology service has made numerous changes in recent years to provide streamlined care for patients, focusing on reduced wait times & improve quality of care. The original pathway was costly in time, involving several waits for the patient: for urgent venepuncture, physician consultation, prescribing of chemotherapy, specialist pharmacist screening of prescriptions & then a separate trip to pharmacy for dispensing. Patients then returned home & waited for a call from the Clinical Nurse Specialist (CNS) to confirm if blood results were appropriate for chemotherapy administration. If a dose adjustment was required the drug was wasted & patients needed to return to hospital for another prescription. Pharmacy waiting times for oral outpatient chemotherapy or supplementary medications are approximately 30 minutes.

**Aims:** We introduced a weekly multi-disciplinary chemotherapy prescribing meeting in 2013 with the aims of improving prescribing safety, minimising time spent prescribing in clinic & reducing patient waiting times. Present at each meeting is a Haematology Specialist Pharmacist, Haematology CNS, Consultant & Specialist Registrar. Chemotherapy is planned a week in advance on ChemCare (an electronic chemotherapy prescribing package). Chemotherapy is prescribed by the attending consultant & immediately screened by the pharmacist; oral chemotherapy is collected from pharmacy by a CNS prior to clinic. All prescription queries are resolved during this meeting. Deferred oral chemotherapy can be returned to pharmacy stock, minimising waste. Intraovarian chemotherapy is pre-planned with authorisation on the day of treatment if the patient is fit to proceed.

**Methods:** In this article to the care pathway, we focused on delivery of care to myeloma patients receiving oral chemotherapy, including setting up a nurse-led clinic. Data have been collected to assess service impact, particularly on patient satisfaction. The latter was assessed using a patient survey. Between July-Dec 2014, 66 patients received oral chemotherapy in the Myeloma Consultant-led clinic, Lenalidomide based regimens accounted for 86% of the oral regimens prescribed. On average, 7 patients per week were on maintenance therapy. During this period 8% of chemotherapy courses were deferred due to low blood counts or side-effects. Drugs were not wasted due to the pharmacy agreement.

**Results:** A patient satisfaction survey was undertaken from Jan-June 2015, post-introduction of the nurse-led clinic paired with the MDT chemotherapy prescribing meeting. Patients were asked about a wide-range of quality parameters. Results showed 89% of patients noted a reduction in wait times & 89% felt they spent less time in consultations as a result. All patients noted they spent more time with the nurse specialist & benefitted from not attending pharmacy. All patients rated the service as more efficient.

**Summary/Conclusions:** The MDT approach to prescribing & dispensing oral chemotherapy & supportive medication has streamlined our way of working & led to greater efficiency for both staff & patients. The new model has changed how patients are seen & assessed and minimised drug wastage, an issue that has helped to double the life expectancy of patients with newly diagnosed multiple myeloma.

**E1480**

**THE IMPLICATIONS OF NON-PROPORTIONAL HAZARDS FOR THE MEASUREMENT OF SURVIVAL BENEFT IN HEALTH TECHNOLOGY ASSESSMENT: CURRENT APPROACHES AND THE ROLE OF RESTRICTED MEAN SURVIVAL TIME**

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**Background:** Median survival and hazard ratios (HR) calculated from Cox proportional hazard (PH) models for progression-free survival (PFS) and overall survival (OS) are principal endpoints in clinical trials. The advent of novel agents, including immuno-oncologics, has seen increasing reports of non-proportional hazards (non-PH). When non-PH are present, it is challenging to evaluate the true clinical significance of survival differences even if the HR is median and the treatment effect measures do not represent the comparative benefit over the full period of observed data. In such situations, additional metrics such as restricted mean survival time (RMST) may be valuable.

**Aims:** To determine current methods used by health technology assessment (HTA) agencies when non-PH are observed in assessments of hematology/oncology drugs, and the extent to which RMST is accepted as an alternative measure of treatment benefit in these circumstances.

**Methods:** Methodological guidelines published by 10 HTA agencies in 8 major developed countries (Australia, Canada, France, Germany, Italy [Emilia Romagna, Veneto], Spain, Sweden, and the United Kingdom [NICE, SMC]) and by international organizations (including the Union for International Cancer Control, UICC; Economic Review, ICER) were reviewed to establish recommended approaches for presenting survival benefit from clinical trials, particularly the use of RMST where non-PH were not reported. To determine how these guidelines are implemented in practice, published HTA reports were examined across the 8 countries for 23 oncology agents (including 4 in hematology) approved by the FDA and EMA since 2014, to identify instances where testing for non-PH was conducted and RMST data reported.

**Results:** Guidelines from only 2 agencies (PBAC in Australia and NICE in the UK) described formal testing for non-PH. Testing was reported in 5 (of 9) NICE assessments and 4 (of 10) PBAC assessments. For the hematology drugs, non-PH testing was conducted in 3 (of 4) NICE assessments; it did not hold in 2 instances. Of the agencies (from France, Germany, Italy and Spain), which focus on comparative clinical benefit, only 1 (GENESIS in Spain) discussed the concept of RMST in its guidelines. Of the 10 assessors in the US (including the pharmaceuticals and economic review agencies) 3 (Genentech, Pfizer, and EMA) have data for assessing RMST. Of the agencies (from Australia, Canada, Sweden, and the UK) which focus on cost-effectiveness, all the TLY in Sweden include RMST in their guidelines; RMST was reported in 13 (of 81) HTA assessments from those countries. Of the 3 hematology drugs where non-PH was tested within the NICE process, all 3 (often plasma cell, where there were no reported RMST utilized during economic model sensitivity analyses). Non-PH is not a widely reported issue in US guidelines; however, the ICER has acknowledged it and PH testing was conducted in both ICER reports in oncology.

**Summary/Conclusions:** Testing for non-PH is not widely reported in clinical trials or incorporated into assessments by HTA agencies except by UK NICE. RMST as a metric to assess OS has played a role in assessing clinical benefit within the context of HTA assessments, although not consistently within countries (across drugs) or across countries (for the same drug), as was seen with the hematology agents. As treatments for cancer expand to new classes and indications, instances of non-PH will likely increase; alternative survival metrics such as RMST may have an increasingly important role to play in describing survival benefit in such cases.
Sickle cell disease

E1482 MONITORING OF CHRONIC HEPATIC DAMAGE IN SICKLE CELL DISEASE: LONGITUDINAL OBSERVATION OF A COHORT OF ADULT PATIENTS

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Background: Sickle cell disease (SCD) is an important cause of hepatic damage which can result in catastrophic consequences as acute hepatic failure and contribute to early mortality. In addition, sickle hepatopathy may be the consequence of SCD’s treatment as liver iron overload or viral hepatitis due multiple blood transfusions that these patients require over their lifetime. Therefore both SCD itself and related therapies may lead幼儿 to fibrosis/cirrhosis.

Aims: We evaluated liver fibrosis using Transient Elastography (TE) in patients with SCD, exploring possible correlation with clinical, laboratory and imaging findings in longitudinal way.

Methods: SCD patients with at least one stiffness evaluation were retrospectively evaluated in the decade 2006-2016 using biochemical markers (liver damage, cholestasis, liver synthetic capacity, iron overload, viral hepatitis and hemolytic index), TE and liver imaging (ultrasound, MRI-R2*).

Results: 37 adult patients were evaluated: 32% HbSS, 68% HbSβ°, median 39yrs, 46% male, median stiffness 6.6 KPa IQR: 5.1-9.1 KPa (Table). There were no differences of stiffness value for gender, genotype. A positive moderate correlation was observed between TE and serum ferritin values (R²=0.43, p=0.008), ALT (R²=0.42, p=0.01), AST (R²=0.49, p=0.0022), conjugated bilirubin (R²=0.59, p<0.001), ALP (R²=0.51, P=0.002); a positive strong correlation was observed between TE and GGT (R²=0.78, p<0.001), negative moderate correlation with the albumin (R²=0.47, p=0.0048). We found that the group of patients on exchange programmes had a value of stiffness lower than the group transfused (p=0.007). No significant correlation was found between stiffness and LIC (R²=0.11, p=0.67). For 24 patients all record were available at time of first observation until last follow up (f.u.): 75% HbSβ°, median age 39.5yrs, male 42%, median f.u. 6 yrs, median stiffness 7.3 KPa IQR: 5.3-11.9 KPa. At the first evaluation we documented a significant positive-moderate correlation of TE with serum ferritin (R²=0.43, p=0.037), AST (R²=0.54, p=0.008), conjugated bilirubin (R²=0.52 values 0.009) and positive-strong correlation with GGT (R²=0.68, p=0.001); these parameters except of ferritin (R²=0.3, p=0.15) and AST (R²=0.39, p=0.058) have maintained the correlation with last f.u.; albumin and ALP showed a significant strong correlation only at f.u. (albumin R²=0.64, p=0.004; ALP R²=0.7, p=0.0017). To remove factors associated with liver fibrosis we also conducted this analysis in the subset of patients HCV negative without liver iron overload: 26 patients, HbSβ° 73%, median age 40.5yrs, male 50%, median f.u. 6 yrs, median values of stiffness 6.1 KPa IQR: 4.6-7.4 KPa. All significant correlations previously described were confirmed also in this group. Three patients in this cohort presented stiffness value according to F4 METAVIR since their first evaluation: all these patients showed pauci-symptomatic disease in terms of VOCs, however they had a severe hepatic damage due to sickle cell disease.

Table 1.

<table>
<thead>
<tr>
<th>Table 1. Predictor variables of interest</th>
<th>&quot;Severe&quot;</th>
<th>&quot;Moderate&quot;</th>
<th>P1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.7 (10.6)</td>
<td>40.9 (12.6)</td>
<td>0.009</td>
</tr>
<tr>
<td>Male sex*</td>
<td>21 (37.5%)</td>
<td>12 (37.5%)</td>
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</tr>
<tr>
<td>Education (years)</td>
<td>13 (1.8)</td>
<td>13 (1.7)</td>
<td>0.80</td>
</tr>
<tr>
<td>Mild Cognitive Impairment*</td>
<td>17 (30.4%)</td>
<td>3 (9.4%)</td>
<td>0.14</td>
</tr>
<tr>
<td>DSST T-score</td>
<td>47 (14.5)</td>
<td>51 (13.4)</td>
<td>0.006</td>
</tr>
<tr>
<td>O2 Saturation (%)</td>
<td>93 (5.8)</td>
<td>98 (1.1)</td>
<td>0.14</td>
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<tr>
<td>WBC count (X 10³/L)</td>
<td>9 (7.3)</td>
<td>9 (2.7)</td>
<td>0.87</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>9.2 (1.5)</td>
<td>11.5 (1.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Platelet count (X 10³/L)</td>
<td>244 (179.8)</td>
<td>283 (115.1)</td>
<td>0.16</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>1.7 (4.0)</td>
<td>1.1 (1.8)</td>
<td>0.51</td>
</tr>
<tr>
<td>LDH (UL/L)</td>
<td>32 (143.3)</td>
<td>289 (149.1)</td>
<td>0.18</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>1118 (1864.4)</td>
<td>404 (1042.2)</td>
<td>0.05</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.7 (0.3)</td>
<td>0.8 (0.2)</td>
<td>0.91</td>
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<tr>
<td>SBP (mm/Hg)</td>
<td>113 (13.4)</td>
<td>118 (13.6)</td>
<td>0.08</td>
</tr>
<tr>
<td>DBP (mm/Hg)</td>
<td>68 (7.8)</td>
<td>73 (19.8)</td>
<td>0.02</td>
</tr>
<tr>
<td>MAP (g/dL)</td>
<td>33 (8.4)</td>
<td>88 (10.0)</td>
<td>0.03</td>
</tr>
<tr>
<td>Hydroxyurea use*</td>
<td>32 (57.1%)</td>
<td>10 (31.2%)</td>
<td>0.04</td>
</tr>
<tr>
<td>Opiate use*</td>
<td>15 (26.8%)</td>
<td>10 (31.2%)</td>
<td>0.57</td>
</tr>
<tr>
<td>Transfusion history*</td>
<td>17 (31.5%)</td>
<td>5 (16.1%)</td>
<td>0.15</td>
</tr>
<tr>
<td>Stroke history‡</td>
<td>10 (18.2%)</td>
<td>2 (6.2%)</td>
<td>0.08</td>
</tr>
</tbody>
</table>

* Mean (SD) unless otherwise noted. † Age-adjusted. 1 Indicates SCI.

Summary/Conclusions: Psychomotor slowing in SCD differs in relationship to genotype; this difference appears unrelated to history of stroke or severity of anemia and other risk factors examined cross-sectionally. Although relatively infrequent, mild cognitive impairment was detectable in patients with a less severe genotype. Longitudinal studies of SCD should include all diseases genotypes, and examine factors that would reduce the risk of cognitive impairment in each subgroup.

Table 1.
Summary/Conclusions: Early identification of chronic hepatic disease sometimes takes on a symptomatic approach due to the occurrence of various clinical manifestations. Detection of significant hepatic damage is crucial for appropriate therapy. The combination of TE with specific serum markers (GGT, ALP, albumin) is a valid tool to early detection of sickle cell anemia.

E1483

MICROSTRUCTURAL ANALYSIS OF RETINO-CHOROID LAYERS USING OPTICAL COHERENCE TOMOGRAPHY IN ADULT PATIENTS WITH SICKLE CELL DISEASE

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Background: Retinopathy is one of the ophthalmological complications of patients with Sickle cell disease (SCD), due to microvascular occlusions; occasionally, proliferative sickle cell retinopathy (PSR) can lead to severe vision loss. Aims: a. to analyze macular alterations in patients with Sickle Cell Disease (SCD) by spectral-domain optical coherence tomography (SD-OCT), using the automated software for retinal segmentation; b. to investigate relationship between OCT abnormalities and the severity of proliferative sickle cell retinopathy (PSR), c. to elucidate the role of potentially contributory systemic factors on the development of macular thinning. Methods: This is a prospective, observational case-control study. Ophthalmological evaluation, fluorescein angiography and SD-OCT were performed. Central and temporal retinal layers were measured by the SD-OCT Automatic Segmentation software. SCD eyes were divided into two groups based on the presence of visible macular thinning areas. Clinical data and blood samples were collected.

Results: Thirty consecutive adult SCD outpatients were studied (median age 38.7±9.89 [M.F 12:18], including 9 patients with Sickle Cell Anemia (SCA), 17 with Sickle Cell β-Thalassemia and 4 HbS/HbC). One out of 59 eyes (1.72%) was considered due to retinal detachment and severe refractive defect. Nineteen out of 59 eyes (32.2%) and 13 out of 30 SCD patients (43%) were noted to have patchy areas of macular thinning on OCT, mostly seen temporally to the fovea. Among these patients, 6 had SCA, 4 had β-Thalassemia and 3 HbS/HbC. More severe PSR was present in 16/59 eyes (29%); the prevalence of temporal macular thinning was higher (10/16) in eyes with more severe PSR (62.5%). Both inner and outer retinal layers thinning of the foveal region and of the central and temporal macula was found in the overall SCD patients compared with normal controls (p<0.0001). SCD eyes with patchy retinal thinning showed significant reduction of inner nuclear layer (INL) and outer plexiform layer (OPL) in the temporal region. Univariate analysis revealed a significant correlation between patchy areas of severe retinal thinning on OCT and SCD need for transfusions, need for chelation, HbF, ferritin, and transferrin saturation (p<0.05). Logistic regression demonstrated an increased risk of retinal thinning when HbF levels were below 7% (odds ratio=10.3, confidence interval 2.8-39.9, p=0.0003). Conclusion: A wide range of variables including coagulation parameters, giving, including previous history of TEE, were associated with retinal thinning.
4.8% (1.6%), positive D-Dimers in 57/59 (96.6%), decreased protein S in 10/61 (16.3%) and decreased protein C in 13/61 (21.3%). NATEM MCF was increased in 27/61 (44.3%) patients while EXTEM MCF was increased in 31/61 (50.8%) patients. Patients with a history of TEE had higher mean values of NATEM-MCF and EXTEM-MCF and those differences were statistically significant (p=0.023, p=0.011 respectively). There was a statistically significant association between the presence of ischemic lesions in brain MRI and the history of TEE (p=0.01). On the contrary the history of ACS was not correlated with the presence of ischemic lesions in MRI. Chronic Hydroxyurea treatment did not correlate with the absence of ischemic findings in brain MRI. Among patients with ischemic lesions those who were already on chronic hydroxyurea treatment had a shorter NATEM-CT compared to patients without treatment. In patients with ischemic lesions in MRI and a history of TEE NATEM-MCF and EXTEM MCF were higher (p=0.03, και 0.03, respectively).

**Summary/Conclusions:** The presence of microschemic encephalopathy is very common in SCD patients and is associated with a history of TEE, which is also frequent in SCD. There seems to be a permanent activation of the coagulation mechanism in SCD. In SCD patients with SCIs and a history of TEE, apart from clotting factors and natural inhibitors there seems to be a contribution of platelets and cellular elements, possibly sickle cells. The impact of chronic hydroxyurea treatment on the pathogenesis of silent infarcts and TEEs needs further evaluation.

**E1486**

**Abstract withdrawn.**

**E1487**

**INVASIVE BACTERIAL INFECTIONS IN Gambian patients with SICKLE CELL ANEMIA in AN ERA of WIDESPREAD PNEUMOCOCCAL and HAEMOPHILUS INFuenZa type b VACCINATION**

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**Background:** Bacterial infections cause significant morbidity and mortality in patients with sickle cell anemia, especially in populations without reliable access to antimicrobial prophylaxis and treatment. The longstanding use of penicillin prophylaxis and vaccination for Streptococcus pneumoniae and Haemophilus influenzae type b in resource-rich settings has minimised the additional risk of invasive bacterial infections associated with sickle cell anemia. However, these interventions are not routinely implemented in much of Africa, despite this region having the greatest burden of disease, with over 80% of people with sickle cell anemia born on the continent. The Gambia has well-established vaccination programmes for pneumococcal and Haemophilus influenzae type b, which is rare in the region. There is little data on the identity of bacterial infections in African sickle cell anemia populations, and we believe (until this study) there were no data from countries with comprehensive vaccination programmes against Streptococcus pneumoniae and Haemophilus influenzae type b.

**Aims:** Primary: to determine the predominant pathogens causing invasive bacterial infections in a population of sickle cell anemia patients admitted to the Medical Research Council Unit Gambia. Secondary: to review the characteristics of this sickle cell anemia population.

**Methods:** A retrospective analysis of the clinical and laboratory records relating to 161 admissions of 126 patients with sickle cell anemia admitted to the Medical Research Council Unit Gambia over a five-year period (between April 2010 and April 2015) when there was high coverage of pneumococcal and Haemophilus influenzae type b vaccination.

**Results:** Pathogenic bacteria were cultured from blood in 11 of the 131 admissions which had blood cultures taken (8.4%, 95% CI 4.5-14.1%). The most frequent organism isolated was *Salmonella typhimurium* (6/11; 54.5%), followed by *Staphylococcus aureus* (2/11; 18.2%) and other enteric Gram-negative pathogens (2/11; 18.2%) and there was one case of *Haemophilus influenzae* non-type b bacteremia (1/11; 9.1%). No cases of bacteremia caused by *Streptococcus pneumoniae* or *Haemophilus influenzae* type b were identified. The most common diagnosis causing the admission was vaso-occlusive crisis (53/161; 32.9%), followed by infective complications including pneumonia (16/161; 9.9%) and osteomyelitis (12/161; 7.5%). The median length of admission was five days and the median age of patients was five years (IQR: 2-13 years). A new diagnosis of sickle cell anemia was made during the admission in just under half of patients.

**Figure 1.**
SUMMARY/CONCLUSIONS: The clinical manifestations of SCD were thought to be associated only with hemoglobin polymerization for a long time. However, recent studies have shown that SCD is a chronic inflammatory disease. The pro-inflammatory cytokines and IGF are in a state of equilibrium in the human body. It has been reported that IGF-1 plays a major role in the production of NO, which is produced in the endothelium and causes a vasodilatory response, and that it increases antioxidant systems and reduces oxidative stress, thereby decreasing inflammation by reducing pro-inflammatory cytokines. In our study, we found that the serum levels of IGF-1, an important growth factor that has not been studied previously in SCD and has recently been evaluated on the effectiveness of treatment, decreased in SCD patients with painful crisis compared to patients in steady state. It was also found that the levels of inflammatory cytokines, evaluated during the same period, such as IL-6 and TNF-α increased. In conclusion, IGF-1 was thought to play a role especially in the pathogenesis of acute inflammation in SCD.

References
3. J. Kanter1,*, J. Mcelligott1
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5. Madrid, Spain, June 22 – 25, 2017
6. haematologica | 2017; 102(s2) | 807
The plasmatic hemoglobin (cell-free hemoglobin - Hb) was measured by using (Bantu / Bantu + HC) and 54 without (Bantu / Bantu - HC), respectively. The patients were divided into two types of the SNP rs7203560 and the intravascular hemolysis in patients with SCA. Aims: To provide a recommendation for newborn screening program for SCD in Italy. Methods: A panel of experts was identified by Italian Society of Thalassemia and Hemoglobinopathies (SITE) and Italian Onco-Hematology Pediatric Association (AIIEOP). The panel has rigorously reviewed the literature (from 1990 to 2016), the existing recommendations/guidelines of other countries where newborn screening already exists, and results for SCD-DH/IU sites that have been included in the HEMOREAD system ( emphasizing Recommendations Assessment, Development and Evaluation) was used to score levels and grades of evidence. The working group produced the draft guideline, and the final version has been revised by external (international) reviewers and the national patients association (UNITED).

Summary/Conclusions: The recommendations for SCD newborn screening program will be an important tool (i) in discussion of strategic newborn screening panel at national level; (ii) to early identify patients to be treated in comprehensive SCD centers and (iii) to produce epidemiological data required for future design of SCD map in Europe.

Background: The incidence of the Sickle Cell Disease (SCD) has increased in Europe because of the high rate of migration from areas in which carriers of the sickle cell allele account for 19-27% of the entire population. Although SCD is endemic in Southern Italy, the recent migration fluxes spread SCD all over Italy with the number of carriers at about 6.5% of the whole population. The distribution of SCD patients has dramatically changed. The large part of resident immigrants are young with an high fertility rate. Neonatal screening combined with timely diagnostic testing, parental education and comprehensive care management reduces morbidity and mortality of SCD. Up to now, a new born screening program for SCD is not active in Italy and only few pilot studies have been carried out (Ballardini E et al. Blood Transfus. 2013 Apr; 11(2): 245-9.; Venturelli D et al., Blood Transfusion 2014; 12: 346-51.; Roffa R et al. Clin Lab 2014; 60 (12): 2089-93).

Aims: To provide a recommendation for newborn screening program for SCD in Italy. Methods: A panel of experts was identified by Italian Society of Thalassemia and Hemoglobinopathies (SITE) and Italian Onco-Hematology Pediatric Association (AIIEOP). The panel has rigorously reviewed the literature (from 1990 to 2016), the existing recommendations/guidelines of other countries where newborn screening already exists, and results for SCD-DH/IU sites that have been included in the HEMOREAD system ( emphasizing Recommendations Assessment, Development and Evaluation) was used to score levels and grades of evidence. The working group produced the draft guideline, and the final version has been revised by external (international) reviewers and the national patients association (UNITED).

Results: The recommendations were divided into five sections according to the newborn screening program as well as: 1) testing of newborn and specific screening methods, 2) evaluation of screening results for a definitive diagnosis, 3) enrollment of affected newborns in comprehensive care programs, 4) evaluations of the efficacy of follow-up and interventions, and assessment of the benefit to the patient, family, and society. The on line access for recommendations will be available for clinicians and healthcare providers.

Summary/Conclusions: The recommendations for SCD newborn screening program will be an important tool (i) in discussion of strategic newborn screening panel at national level; (ii) to early identify patients to be treated in comprehensive SCD centers and (iii) to produce epidemiological data required for future design of SCD map in Europe.

E1492
GENETIC HEMOLYTIC MARKER IN SICKLE CELL ANAEMIA
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Background: The heterogeneity and complexity of the phenotypic profile among individuals with sickle cell anemia (SCA) its one of the principal factors of current research. The SCA, a haemolytic anaemia with severe clinical consequences. The intravascular hemolysis is a chronic clinical subphenotype and has been associated as an independent risk factor related to complications such as pulmonary hypertension, leg ulcer and more recently with progression of vasculopathies. Researches has already shown that the heterogeneity of the hemolytic profile can be due to the presence of different beta S-globin gene cluster haplotypes among the individuals, which suggests the participation of genetic factors in the characterization of this subphenotype. Thus, search for genetic variants has been a promising strategy to assist in the individualization of treatments, and favoring clinical evolution. Recent studies showed that the presence of at least one rs7203560 SNP allele of the SNP rs7203560 and the intravascular hemolysis in patients with SCA. Aims: Our objective were to evaluate the association between different genotypes of the SNP rs7203560 and the intravascular hemolysis in patients with SCA.

Methods: We evaluated 76 Brazilian people with SCA, all with a Bantu / Bantu haplotype profile, and in a steady state. The patients were divided into two groups depending on the SNP rs7203560 (HC) 22 (HC: 0.47) and 54 using (Bantu / Bantu + HC) and 54 without (Bantu / Bantu - HC), respectively. The plasmatic hemoglobin (cell-free hemoglobin - Hb) was measured by enzyme-linked immunosorbent assay (ELISA) to evaluate intravascular hemolysis. The association between categorical variables (with or without use of HC and genotypes SNP genotypes) and cell-free Hb levels was performed by univariate covariance analysis (GLM), followed by Fisher’s Post Hoc, considering the gender and age covariates. Statistical software was used and assumed p <0.05 as significant.

Results: E1493
ASSESSMENT OF INTERNATIONAL DAY HOSPITALS/INFUSION UNITS FOR THE EVALUATION AND TREATMENT OF SICKLE CELL DISEASE
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Background: A Sickle Cell Disease (SCD) Day Hospital is defined as a “dedicated facility for the treatment of SCD uncomplicated painful crises, operating on principle-based pain management”. SCD Day Hospital/Infusion Units (SCD-DH/IU) play a positive role in improving pain management, preventing emergency room visits, hospitalizations, and readmissions. No study to date has systematically surveyed the availability, organization, diagnostic tools/therapy provided, and number of SCD patients treated at these facilities as well as compared these facilities’ practices based on location.

Aims: To evaluated and compare availability and characteristics of key SCD-DH/IU components with the overarching goal of enhancing and standardizing across facilities, guidelines and standard of care and help supporting the development of alike outpatient-care units at other health care institutions and countries.

Methods: A Web-based survey was developed and link to the survey sent via email in September 2016 and January 2017, to 120 health care providers (80 in the USA and 40 in other countries) identified the Global Sickle Cell Disease Network as caring for individuals with SCD. Responses were collected between September 4 and February 10, 2017. Data was analyzed by descriptive statistics and T tests using Graphpad.

Results: Fifty seven surveys were completed (41% response rate) from 51 unique institutions. Responses from the USA sites, 27 (53%) were, mostly, from long-standing sickle cell institutions in the East, West, and South. Non USA sites, 15 (29%) included Canada, Oman, France, Kuwait, and England. Location of nine sites (18%) was not available. Data from only 42 sites showed: 34 (81%) sites reported having SCD-DH/IU facility. Thirty-one (73%) sites care for 200 or more individuals with SCD, including 17(40%) caring for more than 400 SCD patients. Self-standing units accounted for 30% of SCD-DH/IU, while most (63%) were part of a multi-speciality unit. Only three sites operated 24 hours/day, 7 days/week, while 50% of the sites functioned Monday-Friday, 8am-5pm. Half of the SCD-DH/IU sites treat 1-3 SCD patients, 31% treated more than 400 SCD patients. Treatments available at SCD-DH/IU varied among sites. All performed blood tests, but not all were able to provide IV hydration, IV pain management, and blood transfusions. SCD-DH/IU data such as utilization, therapy outcomes, and admissions/readmissions were provided for 63% of the sites, 74% of the sites only 44% have standard post-discharge/follow-up procedures, ¼ of those were Non-USA sites. Most (69%) sites provide individualized care plans for pain management. Only 29% use Patient Controlled Analgesia (PCA). Most 85% allowed direct hospital admission for patients initially evaluated in the SCD-DH/IU. Seven (19%) sites do not have a dedicated provider (MD/PNP) available to triage SCD patients presenting to the SCD-DH/IU, while 71% of the sites may have a dedicated provider. Few (4%) sites provided a dedicated provider to address patients’ psychosocial issues, 21 sites have only a SW, one has only a psychologist, and six neither available. When presented with three different clinical scenarios, sites significantly differed in services availability. Data analyzed based on geographical location, i.e. USA vs Non-USA showed similar trends for high usage of data tracking in the USA sites; but higher availability of triage medical staff in Non-USA sites. Notably, 50% of Non USA sites, only treated patients 18 years and younger, p<0.003.
**Table 1.**

<table>
<thead>
<tr>
<th>Material</th>
<th>Description</th>
<th>Code</th>
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<tbody>
<tr>
<td>Table 1</td>
<td>Summary/Conclusions: This is the first study highlighting key healthcare practice data for the small but significant number of SCD Day Hospital/Infusion Units around the globe. Our data suggest that among institutions with SCD-DH/IU there is no consensus regarding clinical practice or data collection. We conclude that there is a significant need to further evaluate SCD DH/IU patient-based value, and to develop operational standards / benchmarks to ensure dissemination, adaptability, and sustainability of these alternative care models.</td>
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<td><strong>E1494</strong></td>
<td><strong>REDUCED SERUM HAEMOPEXIN LEVELS IN HAEMOGLOBIN SC DISEASE OCCUR INDEPENDENTLY FROM THE DEGREE OF HAEMOLYSIS</strong></td>
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<td><strong>F. Vendrame1,1, L. Marani1, S. Saad1, F. Costa1, K. Kerwin1</strong></td>
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<td>1Hematology and Hemotherapy Center, Unicamp, Campinas, Brazil</td>
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<td><strong>Background:</strong> In intravascular haemolysis, saturation of haptoglobin leads to haemoglobin oxidation and the release of free haem, whose main scavenger is haemopexin. In sickle cell mice, excess free haem has been shown to cause vaso-occlusion that can be reversed by haemopexin, implicating that knowledge on how haemolysis changes haemoglobin production may influence the applicability of clinical use of haemopexin for sickle cell disease and other haemolytic states. Recent studies have reported reduced haemopexin levels in children with sickle cell disease (Santiago et al., 2016) and adults with beta thalassemia (Vinci et al., 2016) in association with elevated haem levels, thus suggesting haemopexin decreases due to chronic haemolysis. No data are available in adults with milder sickling disorder haemoglobin SC (HbSC) disease.</td>
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<td><strong>Aims:</strong> In this study, we examined haemolytic markers, haem, and haemopexin levels in samples from HbSC patients with varying degrees of haemolysis in comparison with healthy subjects with no abnormal haemoglobins (HbAA group).</td>
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<td><strong>Methods:</strong> Forty HbSC patients (age range 25-68 years, 15 men) and forty HbAA controls (age range 18-66 years, 28 men) participated in this study. Exclusion criteria were pregnancy, other cause of haemolysis, history of blood transfusion or sickle cell pain crisis in the past 3 months. Venous blood samples were collected for complete blood counts (Advia 2120, Siemens) and measurement of lactate dehydrogenase (LDH), bilirubin (Roche Hitachi), haem (Bioassay Systems), and haemopexin (Abcam) levels. Statistical analysis was performed with GraphPad Prism v5 and data are expressed as mean±standard deviation.</td>
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<td><strong>Results:</strong> As expected, serum LDH, total and indirect bilirubin, and reticulocyte counts were increased in HbSC patients (P&lt;0.0001). Despite this, no significant difference in total circulating haem was found between HbSC and HbAA (39±2.6 vs 35±1.8 μM, respectively, P&gt;0.30), contrary to what has been reported in other haemolytic diseases. Haemoglobin (Hb) was higher in the HbAA group when compared to the HbSC group (15±0.2 vs 12±0.3 g/dL, respectively, P&gt;0.30), contrary to what has been reported in other haemolytic diseases. No significant difference was found between HbSC and HbAA patients infected by EB as compared to NIP+IP with other known etiological agents [51% vs 85%, OR=0.19, 95%CI=0.08-0.44, Pc&lt;0.003]. Other TLR genotypes apparently confer protection in NIP+IP and in particular TLR-2 rs4969480 TA genotype was significantly less frequent in the group of patients infected by EB as compared to NIP+IP with other known etiological agents [51% vs 85%, OR&lt;0.19, 95%CI=0.08-0.44, Pc&lt;0.003]. Other TLR SNPs, genotype and haplotype showed no significant difference between groups.</td>
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<td><strong>Summary/Conclusions:</strong> Although malnutrition and inflammation are known risk factors for infection, the literature shows the importance of the innate immune response in SCD. This study aimed to explore the relationship between haemopexin levels and the innate immune response in SCD patients. Our results suggest that reduced haemopexin levels are associated with an increased risk of infection in SCD patients. These findings highlight the potential role of haemopexin in the pathophysiology of sickle cell disease and suggest that targeting haemopexin levels may be a promising therapeutic strategy for reducing the risk of infection in SCD patients.</td>
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**E1495**

**ASSOCIATION OF TOLL-LIKE RECEPTOR 2 GENE POLYMORPHISM WITH THE INCIDENCE OF BACTERIAL INFECTIONS IN SICKLE CELL DISEASE**

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1Eurocord, Université Paris 7, Paris, France, 2Monacord, International Observatory on Sickle Cell Disease, Centre Scientifique de Monaco, Monaco, Monaco, 3Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil, 4Hôpital Tenon, 5Inserm U1160, Université Paris 7, Paris, 6Hôpital Henri Mondor, Créteil, France, 7Pediatrics Unit, Centre Hospitalier National d’Enfants Albert Royer, Dakar, Senegal

**Background:** Despite antimicrobial prophylaxis and immunization, bacterial infection remains a leading cause of morbidity and mortality in sickle cell disease (SCD) patients. Functional hyposplenemia/asplenia partially explains their susceptibility, as similar patients with a functional spleen are at raised infectious risk. Toll-like receptors (TLR), that recognize pathogen molecular patterns, are at the forefront of immune protection. The interaction between TLR and infectious diseases in SCD patients has never been explored.

**Aims:** To evaluate if functional polymorphisms in TLR confer susceptibility/resistance to infections in SCD.

**Methods:** 160 SCD patients followed either in France (n=104) or Senegal (n=56) with recorded history of infections were tested for SNPs in TLR-1, TLR-2, TLR-4, TLR-6 and TLR-10 by TaqMan S-nuclease assay for their association with infectious history. Comparisons between groups were evaluated by x² or Fisher exact T-test with Bonferroni corrections of P-value (Pc); associations were measured by odds ratio (OR).

**Results:** 70 patients were positive for at least one bacterial infectious episode (IP) and 84 had no infection (NIP). Eleven IP had more than one episode of infection. Median age was 25 years (range 4-49) for IP and 23 years (range 3-52) for NIP with no distribution bias in gender (p=0.24). All patients had vaccinations against Streptococcus pneumoniae and Haemophilus influenza B, and patients under 10 years had received penicillin prophylaxis. Etiological agent was identified in 58 cases with encapsulated bacteria (EB) occurring in 35; the most common agents consisted of Mycobacterium tuberculosis, Streptococcus pneumoniae, Salmonella spp, Escherichia coli and Klebsiella pneumoniae. Sites of infection included respiratory tract (n=24), bone and joints (n=21), blood stream (n=17), urinary tract (n=11), central nervous system (n=8) and abdominal (n=5). TLR-2 rs4969480 TA genotype was less represented in IP than in NIP [45% vs 98%, OR=0.02, 95%CI=0.01-0.09, Pc<0.003] and in particular TLR-2 rs4969480 TA genotype was significantly less frequent in the group of patients infected by EB as compared to NIP+IP with other known etiological agents [51% vs 85%, OR<0.19, 95%CI=0.08-0.44, Pc<0.003]. Other TLR SNPs, genotype and haplotype showed no significant difference between groups.

**Summary/Conclusions:** TLR polymorphisms apparently confer protection against infections especially for EB. Given the previously demonstrated association of AA genotype with exacerbated expression of inflammatory cytokines as well as association of T allele with lower expression of cytokines, it is tempting to postulate that TA genotype can be considered as a compromise between deleterious effects of over inflammatory response (TLR-2 AA genotype) and under response (TLR-2 TT genotype) to infectious agents. Such balanced selection effect is probably reflected by the observed deviation from HWE.
Stem cell transplantation - Clinical

E1496
HIGH PROGNOSTIC VALUE OF PRE-SCT MOLECULAR MINIMAL RESIDUAL DISEASE ASSESSMENT BY WT1 GENE EXPRESSION IN AML TRANSPLANTED IN CYTOLOGIC COMPLETE REMISSION
A. Candoni1, M. Zaninieri1, E. Bertoli1, F. De Marchi1, E. Simone1, C. Fili1, D. Lazzarotto1, G. Venturi1, E. Tomolli1, N. Rabassi1, C. D’Odonco1, C. Comuzzi1, R. Fanin1
1Division of Hematology and SCT, University Hospital, Udine, Udine, Italy

Aims: We analyzed the outcome of allogeneic Stem Cell Transplantation (allo-SCT) in AML patients according to molecular Minimal Residual Disease (MRD) at the pre transplantation (pre-SCT) workup, assessed by the quantitative expression evaluation of the panleukemic marker Wilm’s tumor gene (WT1), according to LeukemiaNET validated method.

Methods: 122 consecutive AML patients received allo-SCT while in cytologic Complete Remission (cCR), between 2005 and 2016, at our Center. The median age at SCT was 53 years (18-70). The quantitative analysis of the WT1 gene expression (bone marrow samples) was available in 100% cases, both at diagnosis (100% overexpressing WT1 with a mean of 8607±8187 copies/10⁴ AML blasts) and before allo-SCT (81±2566 MRD-WT1-negative and 41±12244 MRD-WT1 positive cases at the pre-SCT workup). We evaluated post-SCT Overall Survival (OS), Disease Free Survival (DFS) and Relapse Rate, according to MRD-WT1 pre-SCT status.

Results: Both post-allo-SCT OS and DFS were significantly better in patients who were MRD-WT1 negative (WT1<250 copies) at the time of SCT compared with those who were MRD-WT1 positive (WT1>250 copies), with a median OS and DFS not reached in the MRD-WT1 negative group and 9 and 8 months, respectively, in the MRD-WT1 positive group (OS log-rank p<0.0001; hazard ratio HR=16.19, 95% confidence interval [95% CI]=0.03-136.3; DFS log-rank p<0.0001; HR=93.73, 95% CI=2.0-6.72). The relapse rate after allo-SCT was 15% (12/81) in pre-SCT MRD-WT1 negative cases and 44% (18/41) in MRD-WT1 positive cases (p=0.00073). At univariate analysis, MRD-WT1 negativity before allo-SCT and grade <2 acute GVHD were significant prognostic factors for improved OS and DFS. However, at multivariate analysis, MRD-WT1 negativity before allo-SCT was the only independent prognostic factor for improved OS and DFS.

Summary/Conclusions: These data show that pre-allo-SCT molecular MRD evaluation through WT1 expression is a powerful predictor of post-SCT outcome (OS, DFS, relapse rate). Patients with both cCR and a MRD-WT1 negativity before allo-SCT have a very good outcome with a very low relapse rate and better survival. The pre-SCT MRD-WT1 stratification in AML is a valuable tool to identify patients, transplanted in cCR, who are at high risk of relapse and who could be considered for conditioning regimen intensification and/or modification for hematological malignancies.

E1497
GOOD IMMUNOLOGICAL RECONSTITUTION IN ADULTS WITH ACUTE LEUKEMIA AFTER ALFA-BETA TCR/CD19+ DEPLETED HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSC-Tx) WITH BUSULFAN, CYCLOPHOSPHAMIDE AND ANTI-THYMOGLOBULIN (ATG) - LINKED T CELL DEPLETION. CURRENT STUDIES TO FOCUS ON THE IN VIVO EFFECTS OF ATG LINKED T CELL DEPLETION
L. Prezioso1,*, S. Bonomini1, C. Schifano1, I. Monti1, I. Manfra1, A. Spolzino1, G. Sammarelli1, L. Craviotto1, M. Sassi1, M. Soli1, F. Aversa1
1Hematology and SCT, University Hospital, Udine, Italy

Background: Haplo-HSCT based on the infusion of high numbers of T cell depleted (TCD) hematopoietic progenitor cells and no post-transplant immunosuppression controls both graft rejection and GVHD in patients with acute leukemia. One major remaining issue is the delay in the post-transplant immunological reconstitution because of the minimal residual T lymphocytes in the graft and in vivo ATG-linked T cell depletion. Current studies are focussing on rebuilding posttransplant immunity to improve clinical outcomes separating GVHD from favourable donor immune responses. Selection of either αβ+ T cells retains in the graft NK, dendritic cells, monocytes and γδ T lymphocytes. Under this approach, a rapid immunological reconstitution and very promising outcome have been reported in pediatric patients.

Aims: With the aims of confirming these results in adults, we tested this approach in adults with acute leukemia.

Methods: Thirty-two patients, median age 51 years (range 19-74), with AML (n=14), ALL (n=11) and AML in first remission (n=7) entered to study. Twenty were in CR (12 CR1; 8 CR2), 12 in advanced-stage disease at transplant. Conditioning consisted of ATG 1.5mg/kg from day -13 to -10, Treosulfan 12 gr/sqm from -9 to -7, Fludarabine 30mg/sqm from -6 to -2 and Thiopeta 5mg/Kg on days -5 and -4. PBPCs from haplo-donor (3 mothers, 9 siblings, 13 sons/daughters and 7 cousins) under underwent in vitro CD19+ depletion by ClinIMACS. No post-transplant immunosuppression was given. Ganciclovir was given over the conditioning regimen in the 22 patients who were CMV seropositive; L-AmB was used as anti-mold active prophylaxis over the neutropenic phase.

Results: Grafts contained a median of 11x10⁶/kg (range 5-19) CD34+ cells, 4.3x10⁵/kg CD3+Tcells/kg (range 1-36), 4.9x10⁵/kg αβ+ T cells, 4x10⁵/kg γδ+Tcells/kg (range 1-34), 5x10⁴B cells/kg (range 1.5-32) and 22x10⁶CD56+NK cells/kg (range 5-91). All patient achieved a full donor sustained engraftment. Median time to reach 500 neutrophils and 20,000 platelets was 13 (range 10-18) and 11 days (range 6-30), respectively. Two patients developed and died from severe acute GVHD. One of them had received the highest dose of αβ+ T cells (3.7x10⁹/kg) and the second one affected by 6GPDH deficiency experienced a late onset hepatic GVHD. Eight patients had skin limited grade II aGVHD that required short course corticosteroids. Only two patients have so far developed mild cGVHD that recovered completely after steroid and cyclosporin treatment. Tending to confirm our working hypothesis, there was a rapid, sustained increase in peripheral blood T-cell subpopulations (Fig. 1). Naïve and memory T-cell subsets increased significantly over the first year after transplantation. B-cell reconstitution was rapid and sustained and immunoglobulin serum levels normalized within 3 months. CMV reactivation occurred in 9 of the 30 patients who were at risk (positive and/or 2 or more CMV reactivations). One with unfavorable serology (donor negative into recipient positive) developed and died of CMV disease 8 months after transplant. Relapse was the main cause of failure (8/12 in relapse, 3/20 in CR). NRM was 15% (4/12 in relapse, 4/20 in CR), 13 patients survive at a median follow-up of 29 months (range 5-53).

E1498
UNMANIPULATED HAPLOIDENTICAL TRANSPLANTATION CONDITIONING WITH BUSULFAN, CYCLOPHOSPHAMIDE AND ANTI-THYMOGLOBULIN FOR ADULT SEVERE APLASTIC ANEMIA: GOOD OUTCOME AND PROGNOSIS ANALYSIS
Z. Xu1, L. Xu1,*, F. Wang1, X. Mo1, T. Han1, W. Han1, Y. Chen1, Y. Zhang1, J. Wang1, Y. Wang1, C. Yan1, Y. Sun1, F. Tang1, X. Zhang1, X. Huang1
1Peking University People’s Hospital, Peking University Institute of Hematology, Beijing, China, Beijing, China

Background: Severe aplastic anemia (SAA) is a life-threatening disorder for which allogeneic hematopoietic stem cell transplantation (HSCT) is the available curative approach. Recently, more and more studies have focused on the feasibility of haplo-identical transplantation in SAA patients because of donor availability. Understanding the outcomes and prognosis of haploidentical hematopoietic stem cell transplantation (HSCT) in adult patients with acquired severe aplastic anemia (SAA), we conducted a retrospective analysis.

Methods: A total of 49 SAA adults received haplo-identical transplantation without in vitro T-cell depletion between May 2011 and December 2016. Of all 47 cases surviving for more than 28 days achieved donor myeloid engraftment. The median time for myeloid engraftment was 13 (range, 10-21) days and for platelet was 17.5 (range, 7-101) days with the cumulative incidence of 93.88±0.17%. The cumulative incidence of grade II-IV and III-IV acute graft-versus-host disease (aGVHD) were 20.89±0.35% and 4.17±0.08%, respectively.
23 patients showed prompt recovery of neutrophils and platelets. So far, despite the infusion of higher numbers of T-cells, no increase of GVHD was apparent. Similar results were seen after both types of mobilization. No graft failures were observed and all identical sibling donors with limited side effects and results in sufficient numbers of CD34+ cells for transplantation. While absolute numbers of CD34+ cells (HSPC) with limited side effects and therefore could be of advantage for allogeneic stem cell donors.

Aims: We set out to address the feasibility of sc PFX in family donors and their recipients. Feasibility was defined by the percentage of HLA-matched sibling donors and recipients with low side effects and therefore could be of advantage for allogeneic stem cell donors. Aims: With the goal of immune system reset, autologous hematopoietic stem cell transplantations have been done in patients with multiple sclerosis (MS).

Aims: With the goal of immune system reset, autologous hematopoietic stem cell transplantations have been done in patients with multiple sclerosis (MS).

Aims: With the goal of immune system reset, autologous hematopoietic stem cell transplantations have been done in patients with multiple sclerosis (MS).

Summary/Conclusions: The cumulative dose of Cy is 200mg/kg.

Results: 80 females and 51 males were included; median age was 47 years. 28 have PPSMs, 42 RRSMs, and 61 SPSSMs. All procedures were started on an outpatient basis and two persons were admitted to the hospital during the procedure. In order to obtain at least 1x10^6/kg viable CD34 cells, one to four apheresis were performed (median 1). Total number of viable CD34+ cells infused ranged between 1 and 9.6x10^6/kg (median 2.2). Patients recovered neutrophils and platelets on median day 9 (range 6 to 12). Two individuals needed red blood cells but none needed platelet transfusions. There were no transplant related deaths and the 125 month overall survival of the patients is 100%. In a subset of 78 persons followed for 3 months or more the EDSS (Expanded Disability Status Scale) was assessed three months after the graft and means diminished from 5.2 to 4.9. The EDSS score improved in 33 patients (42.3%), remained stable in 29 (37.1%) and worsened in 16 (20.5%). Best results of EDSS were found in Relapsing Remitting (82%) and Primary Progressive (80%) type of MS compared to Secondary Progressive (71.4%).

Figure 1.

Summary/Conclusions: It is possible to conduct autotransplants for patients with MS employing non-frozen peripheral blood stem cells and outpatient condition. Additional information is needed to assess the efficacy of these procedures in the treatment of patients with MS.

E1501

VEDOLIZUMAB IN STEROID REFRACTORY INTESTINAL GRAFT-VERSUS-HOST DISEASE

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Background: Steroid refractory intestinal graft-versus-host-disease (GVHD) is a critical complication after hematopoietic stem cell transplantation (HSCT), and treatment options are limited. We have previously described successful treatment of this condition with the antibody vedolizumab, targeting the homing of allogeneic T-cells to the intestinal mucosa by inhibiting the binding of T-cell integrin α4β7 to mucosal addressin MadCAM-1.

Aims: Explore outcome of all patients treated with vedolizumab in our department.

Methods: Prospective case series of 13 patients with steroid refractory gastrointestinal GVHD. Patients received 300mg of intravenous vedolizumab at weeks 0, 2 and 6, followed by infusions every 8 weeks if deemed necessary.
Patients were endoscopically evaluated at time of GVHD diagnosis and follow-up. Treatment characteristics are provided in Table 1.

**Results:** All 13 patients experienced clinical responses, which were confirmed by endoscopies and in mucosal biopsies. 10 patients (77%) achieved a clinical response within 28 days, and half of these were complete responses. At last follow-up 10 patients (77%) had achieved sustained complete responses, 2 patients (15%) had responded partially and 1 patient (8%) suffered disease progression. 7 patients (54%) were alive after a median follow up of 35 weeks. The causes of death were transplantation related toxicity, GVHD in other target organs and infectious complications. Increased relative counts of CD25++ CD127low regulatory T-cells prior to treatment were observed in peripheral blood of 7 of 9 evaluable patients, and the relative counts decreased in all 7 patients during follow-up.

**Table 1.**

<table>
<thead>
<tr>
<th>Age, median (range)</th>
<th>60 (48-72)</th>
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<tr>
<td>From allo-SCT to intestinal GVHD, median, days</td>
<td>14 (6-57)</td>
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<tr>
<td>Intestinal GVHD grade prior to moribund, mean</td>
<td>2.1 (4-7)</td>
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<tr>
<td>Histological GVHD grade prior to moriand, mean</td>
<td>1.8 (0-4)</td>
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<tr>
<td>Doses of prednisone, mean (mg/kg/day)</td>
<td>2.2 (1.3-3.0)</td>
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<tr>
<td>Observation time, median (range)</td>
<td>35 weeks (12-90)</td>
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**Summary/Conclusions:** Our findings suggest that vedolizumab may effectively treat steroid refractory cases of intestinal GVHD and is well tolerated. The mechanism of action is believed to be inhibition of allo-reactive T-cells interacting with intestinal endothelial cells. It is unclear why regulatory T-cells were initially increased in our steroid refractory GVHD patients and subsequently normalized. This might initiate a response to the alloreactive inflammation and subsequent redistribution to affected tissues and/or its resolution after successful treatment.

**E1502**

**RISK FACTORS, OUTCOMES AND CHARACTERIZATION OF ‘AUTOLOGOUS GRAFT VERSUS HOST DISEASE’: THE MAYO CLINIC EXPERIENCE**

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**Background:** Graft versus Host Disease (GVHD) is a common complication of autologous stem cell transplantation (SCT) which is caused by the recognition of recipient antigens by the donor T lymphocytes. Acute GVHD remains a major cause of morbidity and mortality and half of the cases are refractory to steroids. The development of GVHD after autologous SCT (ASCT) is a poorly understood phenomenon. While some experts suggest that such an entity does not exist, some ASCT recipients develop clinical and histopathological changes similar to GVHD after autologeneic SCT.

**Aims:** In this analysis, we aimed to elucidate the factors that affect the outcomes of patients with autologous GVHD.

**Methods:** This is a retrospective analysis of patients that received ASCT at Mayo Clinic between January 2006 and December 2016. Autologous GVHD was defined as the development of clinical and histopathological findings indicative of GVHD in ASCT recipients, as determined by pathology review. Survival was estimated and compared using the Kaplan Meier and Log rank tests. The study was approved by the institutional review board.

**Results:** Between 2006 and 2015, 3,891 consecutive patients underwent ASCT. Of these, 35 patients (0.9%) developed symptoms suggestive of GVHD warranting biopsies. In 19 of these 35 patients (54%), the histopathological changes were consistent with GVHD. The most common underlying disease in patients with developed GVHD was multiple myeloma (14 patients, 73.7%) and the most common conditioning regimen used was melphalan (16 patients, 42.1%) and liver involvement in 2 patients (10.5%). The median time to symptom onset was 11 days (range 3-80) and the median time to GVHD diagnosis was 12 days (range 2-162). Most patients (14, 73.7%) had grade 3 or 4 GVHD and the clinical grading correlated with the histopathologic grading in all patients. The median number of prior therapeutic regimens was 2 (range 1-7). GVHD man-...
BASELINE CREATININE CLEARANCE AND ALBUMIN ARE POWERFUL RISK FACTORS FOR ALLOGENEIC TRANSPLANTATION RELATED MORTALITY

R. Shouval1,2, N. de Jong3, J. Fein1, E. Braakman2, A. Broers2, J. Kuipers2, J. Cornelissen2, M. Gobbi1, M. Miglino1, C. Di Grazia2, A. M. Raiola2, R. M. Lemoli1, M. Gobbi1, M. Miglino1, R. M. Lemoli1, M. Gobbi1, A. Balagurov2, R. Shouval1,*

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Background: The course following allogeneic hematopoietic stem cell transplantation (HSCT) varies between individuals. Baseline comorbidities, commonly scored by the Hematopoietic Cell Transplantation-Comorbidity Index (HCT-CI), are important determinants of transplant risk. However, their prognostic utility varies and only partially accounts for transplantation-related mortality (TRM). Standard pre-HSCT laboratory carries objective physiologic information which can be used for TRM risk estimation.

Aims: Determine the value of pre-HSCT estimated creatinine clearance (CrCl), albumin, and alkaline phosphatase (Alk-p) for TRM prediction.

Methods: The study population included 1,217 patients from two European centers. Indications for transplantation and conditioning regimens were diverse. Donors were either HLA-matched sibling donors (54%), matched unrelated donors (30%), or 9/10 HLA-mismatched unrelated donors (15%).

Results: Patients had a median age of 55 years and HCT-CI scores of 0 (24%), 1-2 (39%), and >3 (37%). A cut-off of CrCl<60 ml/min, albumin<3.5 g/dl, and Alk-p>180 IU/l corresponded with 8.8%, 8.3%, and 6.5% of the population, respectively. CrCl and albumin were associated with increased risk and higher cumulative incidence of day-100 and 2-year TRM, regardless of whether they were continuous or categorized (Figure-panel a). A similar pattern was observed with Alk-p, except for day-100 TRM. In a multivariate analysis, a CrCl<60 ml/min and albumin<3.5 g/dl were consistently among the top risk factors for early and late term TRM. Hazard ratios for 2-year TRM of CrCl<60 ml/min and albumin<3.5 g/dl were 2.00 (1.37-2.95) and 2.329 (1.58-3.43), respectively.

Summary/Conclusions: Simple biomarkers can improve pre-transplant risk stratification and potentially be used as a tool for treatment personalization.

E1504

CYTOGENETIC AND MOLECULAR RISK FACTORS AT DIAGNOSIS ARE OVERCOME BY WT1 AND FLOW CYTOMETRY-BASED PRE TRANSPLANT MINIMAL RESIDUAL DISEASE ASSESSMENT IN ADVANCED ACUTE MYELOID LEUKEMIA PATIENTS

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Background: Allogeneic bone marrow transplantation (BMT) offers the only chance of cure for patients with advanced acute myeloid leukemia (AML). High levels of pre BMT minimal residual disease (MRD) have been reported to predict relapse risk in patient transplanted in first complete remission (CR). WT1 expression levels and multicolor flow cytometry (MFC) are the most common tools to evaluate MRD.

Aims: Here, we analyzed the role of pre-BMT MRD assessment as predictor for the post-transplant relapse risk in patient transplanted beyond first CR.

Methods: We retrospectively analyzed the outcome of 92 consecutive AML patients receiving allo-BMT in 2nd (CR2) or 3rd (CR3). Pre-BMT MRD was evaluated by WT1 expression and MFC. Median age at transplant was 45 years. Disease phase was CR2 in 63 patients (68%) and CR3 in 29 (32%). Risk group according to European Leukemia Net (ELN) at diagnosis was low in 28 patients (30%), intermediate in 44 (48%) and high in 20 (22%). Sixty-six patients (71%) received myeloablative conditioning, whereas 26 (29%) were reduced intensity conditioning (haplo-HAPLO) in 24 (26%) and alternative donor in 50 (54%). Median follow-up was 64 months (95% CI 39.8-88.2 months).

A positive MRD FMC was defined by the presence of at least 1x10^9 residual leukemic cells at four or eight since 2011 color flow-cytometry. WT1 copy number at diagnosis and at relapse was assessed by fluorescence in-situ hybridization (FISH).

Results: Patients had a median age of 55 years and HCT-CI scores of 0 (24%), 1-2 (39%), and >3 (37%). A cut-off of CrCl<60 ml/min, albumin<3.5 g/dl, and Alk-p>180 IU/l corresponded with 8.8%, 8.3%, and 6.5% of the population, respectively. CrCl and albumin were associated with increased risk and higher cumulative incidence of day-100 and 2-year TRM, regardless of whether they were continuous or categorized (Figure-panel a). A similar pattern was observed with Alk-p, except for day-100 TRM. In a multivariate analysis, a CrCl<60 ml/min and albumin<3.5 g/dl were consistently among the top risk factors for early and late term TRM. Hazard ratios for 2-year TRM of CrCl<60 ml/min and albumin<3.5 g/dl were 2.00 (1.37-2.95) and 2.329 (1.58-3.43), respectively.

Interestingly, age did not meet statistical significance in models incorporating these biomarkers, suggesting they strongly reflect patients’ physiological status. Alk-p was dropped out in the multivariate analysis. Prediction models for day-100 and 2-years TRM, based only on HCT-CI, had AUCs of 56.4 and 58.6, respectively. The introduction of both albumin and CrCl, separately or combined, resulted in incremental improvement in AUC, topping at 66.1 (+17% increase) and 63.2 (+8% increase), for day-100 and 2-years TRM, respectively (Figure-panel b). The improvement was maintained in all conditioning and donor subgroups.

Summary/Conclusions: Pre-transplantation CrCl and albumin are powerful risk factors for TRM. Deviations from normal ranges were frequent in our cohort, making them useful prognostic markers. We report for the first time the role of CrCl in HSCT prognostication, rather than the traditional HCT-CI cut-off of Creatinine >2mg/dL, which is rare in HSCT population (<1% in our cohort). We also corroborate albumin’s important prognostic role. Incorporation of these simple biomarkers can improve pre-transplant risk stratification and potentially be used as a tool for treatment personalization.
number/Abl copy number 250x10^4 was used as cut-off value for abnormal WT1 expression.

Results: Relapse occurred in 30 patients (33%) and two years non-relapse mortality was 29%. Three-year estimate of OS was 47.9% (median 19 months). The survival probability was significantly affected by donor source (better for HAPLO, p<0.05), ELN at diagnosis (better for ELN low risk, p<0.01), MRD status before BMT and pre transplant MRD evaluated by both WT1 and MFC on bone marrow samples is a reliable predictor of relapse risk and OS which can overcome the ELN risk stratification at diagnosis. Pre BMT MRD negative patients had a significantly better OS, compared with MRD positive ones. MRD positive patients showed an increased risk of relapse, irrespectively of having a low ELN risk at diagnosis. In patients undergoing BMT beyond CR1 pre-BMT MRD status confirms its prognostic relevance and may help in selecting stem cell source. Pre-BMT MRD evaluation may also help in choosing pre-emptive therapeutic strategies.

E1506 IMPACT OF ALLELE SPECIFIC PATIENT:DONOR HLA DISPARITY ON OUTCOME OF REDUCED INTENSITY TRANSPLANTATIONS PERFORMED USING HLA MISMATCHED UNRELATED DONORS: ON BEHALF OF THE ALWP OF THE EBMT


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Background: Allogeneic stem cell transplantation (allo-SCT) represents an increasingly important curative treatment strategy in adults with acute myeloid leukemia (AML), consequent upon both the increased availability of unrelated donors and the advent of reduced intensity conditioning (RIC) regimens. Although optimal outcomes are achieved in patients transplanted using an unrelated donor matched at 10/10 HLA-A, B, CR1, DQB1 alleles it remains the case that many undergo transplantation using a donor matched at only 9/10 HLA alleles.

Aims: There are limited data concerning the impact of specific HLA mismatches on patient outcome and we therefore interrogated the BMTC database in order to characterize the impact of mismatch on transplant outcome.

Methods: 937 patients with AML in CR1 or CR 2 underwent transplantation utilizing a RIC regimen using a 9/10 mismatched unrelated donor between 2001-2015. Of these 264 were transplanted using a donor mismatched at HLA-A, 127 were mismatched at HLA-B, 292 mismatched at HLA-C, 180 mismatched at HLA-DQ and 74 mismatched at HLA-DRB1. 85% of patients received in vivo T cell depletion.

Results: The 2 year leukemia free survival (LFS) for the whole cohort was 45% and the 2 year overall survival (OS) was 50%. The corresponding non- relapse mortality was 26%. Among the patients transplanted from donors mismatched at HLA-A and mismatched at HLA-B the relapse incidence was 23% and 29% respectively. The 2 year relapse mortality was 29%. Three-year estimate of OS was 47.9% (median 19 months). Among patients transplanted from donors mismatched at HLA-A, B, CRD1 and DQB1 the relapse incidence was 23%. The 2 year relapse mortality was 26%. Among patients transplanted from donors mismatched at HLA-A and mismatched at HLA-B the relapse incidence was 23% and 29% respectively. The 2 year relapse mortality was 29%. Three-year estimate of OS was 47.9% (median 19 months). Among patients transplanted from donors mismatched at HLA-A, B, CRD1 and DQB1 the relapse incidence was 23%.

Discussion: HLA-A, B and DQB1 mismatches were associated with increased relapse risk. Results: Relapse still remains the most frequent cause of treatment failure and mortality. According to the data of CIBMTR, relapse has become the leading cause of death following allo-HSCT. The high risk patients with more advanced disease, have a relapse rate of 40–80%. Therefore, prevention and treatment of relapse post allo-HSCT is the most likely approach to improve survival of these patients. It is well known that IFN-α had been widely used in the field of antitumor. Recently it is shown that IFN-α also play an important role in immune modulation to enhance the effect of GVL.

Aims: To determine the efficacy and safety of IFN-α-2b pre-emptive therapy for acute leukemia(AL) patients with relapsing tendencies after allogeneic hematopoietic stem cell transplantation (allo-HSCT).

Methods: Retrospectively analyzed 986 acute leukemia patients undergoing allo-HSCT from Jan .2006 to Mar .2014 in our hospital. After allo-HSCT, 986 AL patients were periodically monitored the minimal residual disease(MRD) including: bone marrow smear, leukemia-associated immunophenotype (LAIP), leukemia specific or related fusion genes, and donor chimerism through multi-parameter detection to evaluate disease status. Patients were given IFN-α-2b 3-2 million units / day by subcutaneous injection for preemptive treatment once a relapse tendency was detected, such as: increasing proportion of blast in bone marrow between 3–5%, or MRD>1.0×10^-3, or leukemia specific fusion gene transfom negative to positive, or dynamic increasing copy number of WT1 more than 200 copies/10^4 abl, or decreasing of donor chimerism(≥ 90%). There were 98 patients who were presented increasing tendency of MRD and were treated preemptively. During the rate of IFN, 114 patients(38.1%) treated with 0-2b therapy, and 67 patients received non-IFN-α-2b therapy such as: withdrawal immunosuppressant, traditional DLI or DC-CIK immunotherapy.

Results: There were no significant differences in disease characteristics between two groups. For the 31 patients who received IFN-α-2b pre-emptive therapy(IFN group), the median time of IFN-αtherapy was 60 days (range: 5–720 days), Twenty five patients had responded to the treatment without progressing to hematological relapse (response rate 80.6%). 2 patients developed to hematological relapse again after temporary response; 3 patients had no response and eventually progressed to hematological relapse. Regarding 66 patients who received non-IFN-α-2b therapy(non-IFN group), the response rate was 67.2%. 20 patients responded to the treatment (RR 32.8%), 45 patients failed to the treatment and progressed to hematological relapse at a median time of 35 (range: 6-940) days. There was significant difference of RR between two group(p=0.000). 31 patients of IFN group tolerate well and no patient terminated therapy due to toxic effects. Among them, 31 patients received IFN-α-2b preemptive therapy, 8 patients 19.4% with aGVHD and 14-45.2% with limited cGVHD. The median follow-up time was 21-4.5-78.5 months. 22 of 31 cases of IFN group maintained disease-free survival. The 5-year overall survival rate (OS) and the leukemia-
free survival rate (LFS) of IFN group were 47.0%±13.9% and 38.7%±13.1% respectively. However, the 5-yr OS and LFS of non IFN group were 14.5%±10.7% and 12.5%±9.4% respectively. The difference were significantly (P=0.000,P=0.002 respectively). Patients with GVHD had significantly better response than patients without GVHD (88.9% vs 53.8%, P=0.043, P < 0.05).

Summary/Conclusions: IFN-α-2b pre-emptive therapy can effectively prevent high-risk patients with relapsing tendencies (1 point) and carries a high risk of mortality. There are currently no standard assessment tools to predict the risk of morbidity and mortality.

Aims: To review the incidence and cause of ICU admission in patients receiving HDC-ASCT and identify pre-transplant factors that may be predictive of transplant morbidity and mortality.

Table 1.

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>N (%)</th>
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<tr>
<td>Mean age (range 15-74)</td>
<td>56.7</td>
</tr>
<tr>
<td>Male (%)</td>
<td>52.2</td>
</tr>
<tr>
<td>Multiple Myeloma (%)</td>
<td>52.9</td>
</tr>
<tr>
<td>Non Hodgkin Lymphoma (%)</td>
<td>31.4</td>
</tr>
<tr>
<td>Hodgkin Lymphoma (%)</td>
<td>15.8</td>
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| Forty-seven percent were in CR, 50% in PR and 3% SD/PD; 14% received three or more chemotherapy lines before transplant (heavily pre-treated). Regarding comorbidities, 62% had low HCT-CI score (score 0), 26% intermediate risk (1-2) and 12% high risk (≥3). Median follow up was 1.1 years (range 100 days–12 years). Early NRM (day 100) was 2.8%, long term NRM (1-3 years) was 4.3-5.8% and OS (1-5 years) was 89-67%. On multivariate analysis risk factors that showed an independent significant impact with NRM and were included in the score were: male patients (1 point), age ≥55 years (1 point), previous chemotherapy (1 point), heavily pre-treated (1 point), HCT-CI ≥3 (1 point) and Non Hodgkin Lymphoma (2 points). One hundred and seventy eight patients (12%) had a score of 0, 2 if it was around 3 (2.6-3.5). The score was significantly associated with early NRM (day 100: 1.1% vs 1.9% vs 9.2 for LR, IR and HR respectively), long term NRM (1-3 years 1.1-1.1% vs 2.9-4.1% vs 15-20%, respectively, p<0.001) (figure 1) and OS (1-5 years 93-78% vs 91-67% vs 73-50% respectively, p<0.001) (figure 2). No significant association was observed with relapse rate.

Methods: All patients receiving HDC-ASCT for myeloma and lymphoma at King’s College Hospital, London between July 2015 and December 2016 were included. Data cut off was 1st February 2017. Electronic patient records were used to collect data on baseline patient characteristics, comorbidities and performance status. The Charlson comorbidity index (CCI) and haematopoietic cell transplantation comorbidity index (HCTCI) were calculated. Univariate analysis of variables was performed using Graph Pad Prism version 5.03. A p value <0.05 was considered significant.

Results: 169 patients received HDC-ASCT. The median age was 58 years (23-74). Patient characteristics are shown in the table (See Image). Thirteen patients (7.6%) required ICU admission at a median of 14 days post cell infusion (range 5-85), with all patients having a neutrophil count <1x10^9/L. The reasons for ICU admission included sepsis (n=12), severe mucositis/colitis (n=11), renal failure (n=7), hypotension and arrhythmias (n=7), respiratory distress (n=4), liver failure (n=1). The median number of days spent in ICU was 9 (range 2-16). Five patients required single organ support (non-invasive ventilation, 2; intropoe support, 2; haemofiltration; 1) and 2 required only management of

Summary/Conclusions: We found that GATMO score had a significant association with long term OS due to an increase in NRM. All end-point risks increased proportionally with the score. This observation should be confirmed in larger series.
fluid balance. Six patients required multi-organ support (non invasive ventilation/ intubation, haemofiltration and inotropic support) and all died. Four patients died within 30 days of HDC-ASCT and had not engrafted neutrophils at the time of death. Two patients died late at day +120 and day +93 post HDC-ASCT. The latter had both successfully engrafted neutrophils but subsequently became neutropenic. Causes of death were neutropenic sepsis (3), cerebrovascular accident (1) and acute pulmonary edema (1) versus host disease (1). By univariate analysis none of the baseline parameters, comorbidities or conditioning regimens were predictive of ICU admission. The only parameter for which there was a trend for significance was baseline cardiac ejection fraction (EF) <50% (p=0.05). Three patients that required ICU had an EF <50% and 2 were on heart failure medications prior to HDC-ASCT. Two of these 3 patients died.

Summary/Conclusions: In this retrospective series, the risk for ICU admission and death following HDC-ASCT was 7.6% and 3.5% respectively. All patients requiring more than one organ support died. The only predictor of ICU admission and death was baseline cardiac function but this would need confirmation in a larger series. Patient selection remains challenging with no definite tool to predict ICU admission or death.

E1510

AUTOLOGOUS STEM CELL TRANSPLANTATION WITH BENDA-EAM (BENDAMUSTINE, ETOPOSIDE, CYTARABINE, MELPHALAN) IN AGGRESSIVE NON HODGKIN AND HODGKIN´S LYMPHOMA

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Background: Autologous Stem Cell Transplantation (ASCT) is standard of care in relapsed diffuse large B-cell lymphoma (DLBCL) and other lymphoproliferative disorders (relapsed Hodgkin´s disease, 1st line mantle cell lymphoma (MCL) or T-cell lymphoma). BCNU, Etopoide, Ara-C, Melphalan (BEAM) is a standard conditioning regimen, but BCNU is known to be associated with interstitial pneumonia (range 2 to 20%) and an increased risk of death compared with other regimens.

Aims: Therefore a less toxic conditioning protocol might improve the results in lymphoma patients. Bendamustine showed promising results in B- and T-cell lymphoma and dose escalation is safe and feasible. Here we report promising results with bendamustine replacing BCNU in the BEAM regimen described as Benda-EAM, previously published in a phase two dose finding study (Visani, Blood 2011).

Methods: Forty-one patients with Hodgkin’s (HL) (n=9) or Non-Hodgkin (n=32) lymphoma were consecutively treated with Benda-EAM (bendamustine on two consecutive days at a dose of 200mg/m² per day). Eleven patients were diagnosed with DLBCL, ten patients with MCL, six patients with follicular lymphoma (FL), three patients with T-cell lymphoma (TCL) and two patients with greyzone lymphoma (GZL). Twenty-seven patients were male and fourteen female with a median age of 52 years (range 22-71) and 25% were above the age of sixty. The median lines of previous therapies were 2 (range 1-4).

Figure 1.

Results: All patients had chemosensitive disease and before transplantation, 34 patients (83%) were in complete (CR) and 7 (17%) in partial remission (PR). A median number of 4.20*10⁶CD34+ cells/kg (range: 1.60-13.30) were infused. All patients showed engraftment with a median time to achieve an absolute neutrophil count >0.5*10⁹/l of 12 days (range 7-110). The median time of fever was 5 days (range: 0-15). The median number of days on G-CSF was 7 (range 4-15) and in median 2 units of red blood cells and 5 units of platelets had to be transfused. The median duration of hospitalization was 25 days. The common grade 3 and 4 toxicity during the whole treatment period was diarrhea (n=10), mucositis (n=7), infections (n=9) and febrile neutropenia (n=6), followed by nausea (n=4) and cardiologic toxicities (n=3). No severe pulmonary or renal toxicities were observed and no transplant related mortality occurred. After a median follow-up of 43 months 22 patients (56%) are still in CR, while 19 patients (44%) became neutropenic. Causes of death were neutropenic sepsis (3), cerebrovascular accident (1) and acute pulmonary edema (1) versus host disease (1). By univariate analysis none of the baseline parameters, comorbidities or conditioning regimens were predictive of ICU admission. The only parameter for which there was a trend for significance was baseline cardiac ejection fraction (EF) <50% (p=0.05). Three patients that required ICU had an EF <50% and 2 were on heart failure medications prior to HDC-ASCT. Two of these 3 patients died.

Summary/Conclusions: In conclusion Benda-EAM is feasible with a quite promising outcome. Currently an international randomized phase II trial comparing Benda-EAM with BEAM is recruiting. So far fifty-five of 110 planned patients are randomized and first results are expected for 2018.

E1511

THROMBOTIC MICROANGIOPATHY WITH CONCOMITANT AGVHD AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: RISK FACTORS, SEVERE OUTCOME AND TREATMENT EXPERIENCE

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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT)- associated thrombotic microangiopathy (TA-TMA) is a significant complication after allo-HSCT. acute graft-versus-host disease (aGVHD) is one of the risk factors for the occurrence of TA-TMA, and some patients may develop both. Although there has been sufficient information available on aGVHD and TA-TMA, TMA with concomitant aGVHD after allo-HSCT remains not well understood.

Aims: To explore the possible risk factors for the occurrence and mortality of TMA with concomitant aGVHD and to investigate outcomes and treatments of this disorder after allo-HSCT.

Methods: This study was based on patients who underwent allo-HSCT at Peking University People’s Hospital from January 2008 to December 2016. We included patients who showed refractory diarrhea and underwent enteroscopy and biopsy. The diagnosis of TA-TMA and aGVHD were mainly based on the probable-TMA criteria (Byung-Sik Cho et al. Transplantation 2010;90:918-926) and endoscopic appearance and histologic findings (Thomas Hematopoietic Cell Transplantation, Fifth Edition, 2016, respectively). The potential factors affecting TMA with concomitant aGVHD occurrence and markers associated with the death of these patients were identified using univariate and multivariate Cox analysis. The cumulative incidence of relapse, non-relapse mortality (NRM), overall survival (OS) and disease-free survival (DFS) were estimated using the Kaplan-Meier method and were compared by the log-rank test.

Results: Among all 3,992 allo-HSCT recipients, 276 patients showed refractory diarrhea and underwent enteroscopy; of these patients, 50 (1.93%) were diagnosed with TMA with concomitant aGVHD and were enrolled in the case group, and 150 (5.80%) were enrolled in the control group. The two groups matched well with regard to baseline characteristics. Based on the nested case-based control study, grade III-IV aGVHD (P=0.000), AKI (P=0.033) and hypertension (P=0.028) were significant independent risk factors associated with the occurrence of TMA with concomitant aGVHD. Considering the case group only, our data suggested that a haptoglobin level below normal (P=0.013), a maximum volume of diarrhea ≥2500 ml/d (P=0.015) and bloody diarrhea (P=0.049) were significant markers for death in both univariate and multivariate analysis. Among the case group and control group, the 9-year OS rates were 52% and 81% (P=0.001), respectively; the 9-year DFS rates were 50% and 65% (P=0.345), respectively; the 9-year cumulative incidence rates of NRM were 44% and 16% (<0.001), and those of relapse were 6% and 19% (P=0.010), respectively. To further study the treatments of patients with TMA and aGVHD, we calculated the OS and found that plasma exchange (PE) use (PE=0, 62.5%; PE ≥0, 36.9%, P=0.156) had no significant influence on the patient outcome.

Summary/Conclusions: This study demonstrated that patients diagnosed with TMA with concomitant aGVHD after allo-HSCT had a significantly lower OS, higher NRM, and a lower incidence of relapse. The risk factors associated with the occurrence and mortality of TMA with concomitant aGVHD may help us assess the prognosis of patients. The findings also suggested that PE use may be ineffective to these patients.

E1512

SIGNIFICANCE OF MINIMAL RESIDUAL DISEASE MONITORING BY QUANTITATIVE RT-PCR IN CORE BINDING FACTOR AML ON TRANSPLANTATION OUTCOMES

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Background: Despite the well-defined role of minimal residual disease (MRD) monitoring in core binding factor (CBF)-AML after intensive chemotherapy, it remains to be seen, to date, a paucity of data assessing the clinical utility of MRD monitoring before allogeneic stem cell transplantation (HSCT).

Aims: We investigated the prognostic impact of MRD monitoring by real-time quantitative polymerase chain reaction (RT-PCR) for RUNX1/RUNX1T1 and year PFS are 73.2% and 57.9% and the 1- and 2-year OVS 85.4% and 79.4%, respectively.

Summary/Conclusions: In conclusion Benda-EAM is feasible with a quite promising outcome. Currently an international randomized phase II trial comparing Benda-EAM with BEAM is recruiting. So far fifty-five of 110 planned patients are randomized and first results are expected for 2018.
CBFB-MYH11 transcript levels at HSCT on transplant outcomes in AML patients with CBF abnormalities.

**Methods:** We included 61 AML patients with CBF at diagnosis that underwent their first HSCT in complete remission (CR) from January 2007 through May 2016. Of 61, 19 (31%) had t(8;21) chromosomal translocation and 42 (69%) inv(16)(p13;q22). Disease status at HSCT was CR1 in 19 (31%) and CR2 in 42 (69%). Disease status at HSCT was based on peripheral blood blasts, bone marrow with dysplastic cells was considered as hypoplastic MDS, and this CA had disappeared again at 79.6 months. None of patients developed MDS or AML after SCT.

**Summary/Conclusions:** This study showed that long-term transplant outcomes in SAA patients with CAs at diagnosis were excellent. Moreover, CAs at diagnosis did not affect the clinical outcome including clonal evolution to other hematologic malignancies after SCT in adult SAA.

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**Figure 1.**

**E1513**

LONG-TERM OUTCOME OF ALLOGENEIC STEM CELL TRANSPLANTATION IN ADULT SEVERE APLASTIC ANEMIA WITH ABNORMAL CYTOGENETICS AT DIAGNOSIS

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1Seoul St. Mary’s Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea, Republic Of

**Background:** Cytogenetic abnormalities (CAs) have been reported at the time of diagnosis of acquired aplastic anemia (AA), up to approximately 4-15%. Considering evolution into clonal hematologic disorders and difficulty between AA and hypoplastic MDS, clinical implications of CAs in AA is important.

**Aims:** In this study, we investigated long-term outcome of allogeneic stem cell transplantation (SCT) in adult severe AA (SAA) patients with abnormal CAs at diagnosis.

**Methods:** Total of 19 patients with abnormal CAs at diagnosis who underwent allogeneic SCT at our institution between 2003 and 2015. Morphologically hypoplastic bone marrow with dysplastic cells was considered as hypoplastic MDS and excluded. CAs were defined as 2 or more cells showing the same chromosomal gain or structural abnormality, or 3 or more cells with the same chromosomal loss.

**Results:** Most frequent abnormality was trisomy 8 (n=11), followed by inversion 9 (n=2). Other CAs included t(3;3), t(5;18), t(11;11), t(1;18), t(1;19), +Y, +Y, -7, +9. Two patients had two or more CAs. Seven male and 12 female patients with a median age of 41 years (range, 20-59 years) were included. Patients had received SCT from HLA-matched sibling (n=12), unrelated (n=5), or haplo-identical donor (n=2). After a median follow-up of 66.3 months (range 12.3-156.3), the 5-year estimated OS rates were 94.7±5.1%. One patient died vs patients (p=0.035). 2-year OS was similar irrespective of PET status (p=0.49). Considering Deauville ≤2 as CMR, there was only a trend towards decreased CIR for metabolically negative scans (p=0.096). Significance of these results remained unchanged after excluding NHL cases. B. Relapse post HCT: Median time to relapse post HCT for patients with CIR ≤2 was 26.8 months (0.6-70.5). Cumulative incidence of relapse (CIR), progression free survival (PFS) and overall survival (OS) at 2 years was 37.9%, 56.1% and 74.8%, respectively. A. PET/CT status pre-HCT. A total of 47 patients had pre-HCT PET/CT and were evaluable for further analysis. Median time from PET to HCT was 17 days (6-59). There were no significant differences between the cohorts based on age at HCT, gender, underlying diagnosis, relapsed/refractory status, time to relapse, number of salvage regimens, number of salvage cycles, use of immunotherapy as part of salvage and post HCT immunotherapy use as maintenance. Considering Deauville ≤3 as complete metabolic response (CMR), 2-year CIR was 16.7% vs 60.5% for PET negative vs PET positive patients (p=0.0021). 2-year PFS was significantly higher in PET negative vs PET positive patients at 72% vs 39.5%, respectively (p=0.035). 2-year OS was similar irrespective of PET status (p=0.49). Considering Deauville ≤2 as CMR, there was only a trend towards decreased CIR for metabolically negative scans (p=0.096). Significance of these results remained unchanged after excluding NHL cases. B. Relapse post HCT: Median time to relapse post HCT for patients with CIR ≤2 was 26.8 months (0.6-70.5). Cumulative incidence of relapse (CIR), progression free survival (PFS) and overall survival (OS) at 2 years was 37.9%, 56.1% and 74.8%, respectively. A. PET/CT status pre-HCT. A total of 47 patients had pre-HCT PET/CT and were evaluable for further analysis. Median time from PET to HCT was 17 days (6-59). There were no significant differences between the cohorts based on age at HCT, gender, underlying diagnosis, relapsed/refractory status, time to relapse, number of salvage regimens, number of salvage cycles, use of immunotherapy as part of salvage and post HCT immunotherapy use as maintenance. Considering Deauville ≤3 as complete metabolic response (CMR), 2-year CIR was 16.7% vs 60.5% for PET negative vs PET positive patients (p=0.0021). 2-year PFS was significantly higher in PET negative vs PET positive patients at 72% vs 39.5%, respectively (p=0.035). 2-year OS was similar irrespective of PET status (p=0.49). Considering Deauville ≤2 as CMR, there was only a trend towards decreased CIR for metabolically negative scans (p=0.096). Significance of these results remained unchanged after excluding NHL cases. B. Relapse post HCT: Median time to relapse post HCT for patients...
E1515

COMPARISON OF OUTCOMES AFTER DONOR LYMPHOCYTE INFUSION WITH OR WITHOUT PRIOR CHEMOTHERAPY FOR MINIMAL RESIDUAL DISEASE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Minimal residual disease (MRD) can predict impending relapse after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Thus, MRD-directed immunotherapy may be a reasonable option for relapse prophylaxis. Despite its potential for tumor lysis, chemotherapy (Chem-DLI) can decrease the tumor burden, and immunotherapy should preferably be started in patients with leukemia with relatively low tumor burden. However, some patients who are MRD-positive may refuse or are unable to receive chemotherapy prior to DLI. Few studies have compared the clinical outcomes of Chem-DLI and DLI alone in patients who were MRD-positive after allo-HSCT.

Aims: The efficacy of DLI without chemotherapy was investigated and compared with that of Chem-DLI in patients who were MRD-positive after allo-HSCT.

Methods: We enrolled 115 consecutive patients who received either DLI (n=20) or Chem-DLI (n=95) during the same period. For each DLI recipient, three recipients matched for age at the HSCT, underlying diseases, and the year of the HSCT were randomly selected from the Chem-DLI cohort (n=60).

Results: The 2-year cumulative incidence of severe acute graft-versus-host disease (GVHD) and chronic GVHD was comparable between the groups. Fifteen (10.3%) patients died (78.3% patients) in the DLI and Chem-DLI groups turned MRD negative, respectively. The 2-year cumulative incidences of relapse and non-relapse mortality after intervention were 30.7% versus 39.6% (P=0.582) and 10.3% versus 6.0% (P=0.508) in the DLI and Chem-DLI groups, respectively. The 2-year probabilities of disease-free, overall, and GVHD-free/relapse-free survival after preemptive intervention were 58.9% versus 54.3% (P=0.862), 69.3% versus 78.1% (P=0.361), and 44.4% versus 35.1% (P=0.489) in the DLI and Chem-DLI groups, respectively. In multivariate analysis, the intervention method did not significantly influence the clinical outcomes.

Summary/Conclusions: In summary, preemptive DLI alone may be effective for patients who are MRD-positive and may be a potential alternative for patients who refuse or are unable to receive Chem-DLI after HSCT.

E1516

DIFFERENTIAL PROGNOSTIC IMPACT OF HEMATOPOIETIC CELL TRANSPLANTATION SPECIFIC COMORBIDITY INDEX (HCT-CI) ON TRANSPLANT OUTCOMES BY STEM CELL SOURCES

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Background: The hematopoietic cell transplantation specific comorbidity index (HCT-CI) has been proposed to predict the probability of nonrelapse mortality (NRM) and treatment-related mortality (TRM) and survival in allo-HCT across transplant centers. However, the impact of HCT-CI on clinical outcomes in single unit umbilical cord blood transplantation (UCBT) has not been investigated extensively.

Aims: The purpose of this single-center retrospective study was to investigate the validity of HCT-CI in UCBT.

Methods: We retrospectively analyzed a cohort of 144 consecutive adult patients who received first allogeneic HSCT between July 2008 and December 2016 in our hospital. One patient was excluded from this analysis due to inadequate data regarding comorbidities before HSCT. Patients were divided into the UCBT group (n=90) or the non-UCBT group (n=53). Two-year OS and 1-year NRM were defined as the primary endpoints.

Results: Pre-transplant parameters, such as gender, diagnosis, and the phase of disease, were comparable between the two groups. The median follow-up durations were 562 days and 627 days for the non-UCBT group and the UCBT group, respectively. The most frequent comorbidity was mild hepatic comorbidity (22%), followed by mild or severe pulmonary comorbidities and active infections (16%). For the non-UCBT group, 2-year OS rates for HCT-CI scores of 0, 1-2 and ≥3 were 70% (n=43), 63% (n=30), and 31% (n=17), respectively (P=0.014). For the non-UCBT group, 1-year NRM rates for HCT-CI scores of 0, 1-2 and ≥3 were 10%, 17%, and 35%, respectively (P=0.026). For the UCBT group, 2-year OS rates for HCT-CI scores of 0, 1-2 and ≥3 were 76% (n=26), 46% (n=13), and 69% (n=14), respectively (P=0.38). For the UCBT group, 1-year NRM rates for HCT-CI scores of 0, 1-2 and ≥3 were 9.0%, 15.7%, and 7.1%, respectively (P=0.75). In multivariate analysis, the HCT-CI score of ≥3 was significantly associated with lower OS (p=0.005; hazard ratio=2.6) and higher NRM (p=0.015; hazard ratio=3.1) for the non-UCBT group, but not for the UCBT group. There was no significant difference in the cumulative incidences of grade 2 to 4 acute GVHD between the non-UCBT group (41%) and the UCBT group (33%; P=0.51). Similarly, there was no significant difference in the cumulative incidences of grade 1 to 4 chronic GVHD between the non-UCBT group (8.8%) and the UCBT group (6.1%; P=0.80). The cumulative incidence of extensive chronic GVHD was significantly higher in the non-UCBT group compared with the UCBT group (38% vs 3.8%; P=0.001).

Although not significant, patients in the non-UCBT group were more likely to have the systemic steroid therapy compared with those in the UCBT group. (54% vs 34%; P=0.084).

E1517

LOW DOSE POSTTRANSPLANTATION CYCLOPHOSPHAMIDE CAN ENHANCE THE PROTECTIVE EFFECT OF ATG/G-CSF ON GVHD: RESULTS OF A PHASE II PROSPECTIVE TRIAL

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Background: Anti-thymocyte globulin (ATG)/granulocyte colony-stimulating factor (G-CSF)-represented regimen produces essentially universal engraftment with limited relapse and favorable survival, albeit with relatively high rates of graft-versus-host disease (GVHD), especially after HCT from maternal donor or collaterals relatives. While use of high-dose, post-transplant cyclophosphamide (PT/Cy) results in low rates of GVHD and favorable immune reconstitution, although with higher rates of relapse and somewhat high rates of graft failure. Thus, novel strategies are needed to refine each approach: under Beijing protocol including ATG and G-CSF, reducing GVHD without abrogating GVL effect is a major priority.

Aims: In order to benefit patients at high risk of developing GVHD without abrogating engraftment and GVL effects, we sought to develop a novel procedure in TCR haplo-HCT with intensified conditioning containing ATG and G-CSF followed by lower-dose of PT/Cy. In addition, the current study attempt to establish a murine model and focus on Treg cells to clarify the immunological mechanisms for GVHD prevention by the new strategy.

Methods: We performed a prospective pilot study of HLA haploidentical cell transplantation, at high risk of developing GVHD with intensified conditioning including G-CSF and ATG, followed by two lower doses of PT/Cy (14.5mg/kg/2x doses; designated as Group A). Outcomes were compared with those of 160 controls from matched-pair analysis who undergo haploidentical HCT from other donors than mother or collateral relatives at the same time period (Group B) as well as with those of 46 historical controls who undergo HCT from mother or collateral relatives at earlier time period (Group C). In addition, the current study attempt to establish a murine model and focus on Treg cells to clarify the immunological mechanisms for GVHD prevention by
E1518

HEPATITIS B REACTIVATION IN HEMATOPOIETIC STEM CELL TRANSPLANTED PATIENTS: 22 YEARS EXPERIENCE OF A SINGLE CENTRE

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Methods: Patient files and electronic records of 561 patients who received HSCT between 1994 and 2015 at the Bone Marrow Transplantation Centre of Cerrahpasa Medical Faculty were retrospectively evaluated. A total of 66 patients with HBsAg (n=15; 12 autologous, 3 alelogenic) and anti-HBc IgG positivity (n=51; 29 autologous, 22 allogenic) were included in the study. Cases were grouped according to transplant types (allogeneic or autologous) and anti-HBc positivity (positive or negative) to calculate relative risks and cumulative incidences of HBV reactivation.

Results: Four (26) of the 15 patients with HBsAg positivity showed HBV reactivation in an average of 13 months following H SCT. While cumulative incidence of reactivation was 7% at day 60, it went up to 16% and 44% at days 270 and 940, respectively. HBsAg positivity was significantly higher in the allogeneic HSCT group (30%; CI: 12%>50%) compared with the autologous HSCT group (0%; CI: 0%>10%). Patients who were anti-HBc IgG positive and anti-HBs negative showed an higher cumulative incidence of reactivation (7% at day 60, 16% at day 270, and 44% at day 940) compared with patients who were anti-HBc IgG positive and anti-HBs positive (0% at day 60, 3% at day 270, and 0% at day 940). The risk of reactivation was significantly higher in patients who were anti-HBc IgG positive (P<0.001 vs CB), and MAC (P<0.001) than CB. Among the clinical variables such as diagnoses (acute leukemia or others), refined disease-risk index (low/intermediate vs high), donor source (MUD, MUD, haplo, or CB), age, first or second HSCT, intensity of conditioning (RIC or MAC), with or without comorbidity, graft failure, GVHD, IV, and admission to the intensive care unit (ICU), multiple regression models revealed that HBsAg positivity (P<0.001), anti-HBc IgG positive (P<0.001), and MAC (P<0.05) were the factors that increased the initial inpatient cost. The transfusion cost was associated with the initial inpatient cost and the length of stay. The mean inpatient cost was €49985 (IQR, 37030-66923), the median transfusion cost was €22750 (IQR, 10200-50000), and the median hospitalization period was 270 days (IQR, 190-450).

Summary/Conclusions: Despite the high prevalence of HBV reactivation in HSCT patients, the risk of reactivation is low in patients with HBsAg positivity. However, patients with HBsAg positivity and HBc IgG positivity should be monitored closely for HBV reactivation, and antiviral prophylaxis should be considered for these patients.

E1519

ALLOGENEIC HEMATOPOIETIC STEM-CELL TRANSPLANTATION FROM HAPLOIDENTICAL DONOR WITH POST-TRANSPLANT CYCLOPHOSPHAMIDE WAS RELATED TO LESS INPATIENT COST COMPARED TO CORD BLOOD TRANSPLANTATION

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Background: The number of allogeneic HSCT from alternative donors such as cord blood (CB) and haploidential donor (haplo) is increasing especially after introduction of post-transplant cyclophosphamide (PT/Cy) as GVHD prophylaxis for haplo. Although comparison of the survival benefit between CB and haplo with PT/Cy has been made by several groups, there is little information about the medical cost and the hospitalization period of HSCT from alternative donors.

Aims: We evaluated the medical costs and the hospitalization period related to allogeneic HSCT in order to clarify the impact of donor sources and other clinical factors on these outcomes.

Methods: Patients (n=134) with hematological malignancies who underwent allogeneic HSCT between January 2013 and December 2016 in University of Tsukuba Hospital were included. The days of the initial hospitalization (from the beginning of the conditioning regimen to discharge), the whole initial inpatient costs and the costs of transfusion during the initial hospitalization were retrospectively analyzed.

Results: The median age of the patients was 46 (range, 16-67) years. The diagnoses were AML (n=66), ALL (n=31), MDS (n=17), lymphoma (n=11), and others (n=9). Twenty-seven patients were transplanted from MRD, 37 from MUD, 22 from haplo haplo with PT/Cy, and 48 with single-unit CB. The median initial inpatient cost was €49179 (IQR, 37030-66923), the median transfusion cost was €11500 (IQR, 9500-15250), and the median length of initial hospitalization was 55 (IQR, 44-75) days. CB showed significantly higher inpatient cost (median, €66852) than haplo (median, €49085, P=0.008 vs CB), MDR (median, €36998, P=0.001 vs CB), and MUD (median, €39262, P=0.001 vs CB). Figure 1. The transfusion cost was highest in CB (median, €22750) compared with haplo (median, €12866, P=0.001 vs CB). Among the clinical variables such as diagnoses (acute leukemia or others), refined disease-risk index (low/intermediate vs high), donor source (MUD, MUD, haplo, or CB), age, first or second HSCT, intensity of conditioning (RIC or MAC), with or without comorbidity, graft failure, GVHD, IV, and admission to the intensive care unit (ICU), multiple regression models revealed that CB (P=0.001 vs MUD), MRD (P=0.001 vs MUD), and MAC (P=0.05) were the factors that increased the initial inpatient cost. The transfusion cost was associated with the initial inpatient cost and the length of stay. The mean inpatient cost was €49179 (IQR, 37030-66923), the median transfusion cost was €22750 (IQR, 10200-50000), and the median hospitalization period was 270 days (IQR, 190-450).

Summary/Conclusions: Although HSCT from alternative donors was related to the higher initial inpatient cost and longer hospitalization, the impact on those outcomes was more significant in CB than haplo with PT/Cy. The higher inpatient cost of CB was attributable to delayed hematological recovery which lead to its larger demand for transfusion. The strategy to improve hematological recovery will be needed to reduce the medical cost especially in CB. The larger scale investigation is necessary for better cost-effectiveness in HSCT.
application after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Peroxisome proliferator-activated receptor (PPAR)-gamma (γ) is a potent anti-inflammatory agent. is a transcription factor belonging to the nuclear hormone receptor super family which may be participating in aGVHD.

Aims: To explore the role of PPARγ in aGVHD after allo-HSCT.

Methods: 65 patients under allo-HSCT and 10 healthy controls were enrolled in study. Peripheral blood (PB) of patients was collected at 15 days, 30 days, 60 days, and 90 days after allo-HSCT. The mRNA expression of PPARγ, IFNγ, T-bet was detected by the real-time PCR. Furthermore, we conducted mixed lymphocyte reaction (MLR) to detect the proliferation of active lymphocytes under different concentration of PPARγ agonist.

Results: Among 65 patients after HSCT, aGVHD occurred in 45 patients. Expression of PPARγ mRNA in healthy controls was significant lower than that in patients after allo-HSCT within 90 days (<0.05). The expression of PPARγ mRNA hold steady in non-GVHD patients within 90 days after allo-HSCT, and was significantly lower in GVHD group than in non-GVHD group (P<0.05). PPARγ expression in severe aGVHD (grade 4) was significantly lower than that in mild aGVHD (grade 1 to 2) patients (P<0.05). The expression of IFNγ and T-bet increased in aGVHD patients and were negatively correlated with PPARγ mRNA expression (<0.05). The experiment of MLR shows that PPARγ agonist rosiglitazone above concentration of 25μM had dose-dependent inhibition effect to proliferation of lymphocytes.

Summary/Conclusions: Low expression of PPARγ is associated with aGVHD occurrence and degree. PPARγ agonist can inhibit the proliferation of lymphocytes, which may be a new way to treat aGVHD.

OUTCOMES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH ACUTE MYELOID LEUKEMIA HARBOURING INV(3)(q21;q26.2)/t(3;3)(q21;q26.2)

E1521

HAPLOIDENTICAL TRANSPLANTATION WITH MYELOABLATIVE CONDITIONING REGIMEN COULD SERVE AS AN OPTIONAL SALVAGE THERAPY FOR YOUNGER PATIENTS WITH REFRACTORY OR RELAPSED NON-HODGKIN LYMPHOMA

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Background: Allogeneic hematopoietic stem-cell transplantation (allo-HSCT) has a well-established role in the treatment of refractory or relapsed (R/R) aggressive non-Hodgkin lymphoma (NHL). However, whether patients with R/R aggressive NHL, in the absence of appropriate HLA-matched donors, can benefit from haploidentical hematopoietic stem cell transplantation (haplo-HSCT) is yet to be elucidated. Herein, we evaluated clinical outcomes of haplo-HSCT in patients with R/R aggressive NHL.

Aims: To evaluated clinical outcomes of haplo-HSCT in patients with R/R aggressive NHL.

Methods: 23 patients with R/R aggressive NHL who had undergone haplo-HSCT in our center between January 2004 and December 2015 were included, and data were retrospectively analyzed. 25 patients with R/R aggressive NHL who received HLA-matched HSCT during the same period constituted the control group for this analysis. All patients received myeloablative conditioning (MAC) regimen in both groups. Likewise, the most important factors that influenced the overall survival rate in the haplo-HSCT group were age and occurrence of grade III–IV aGVHD.

Summary/Conclusions: Haplo-HSCT with MAC regimen could serve as an optional salvage therapy with outcomes comparable those of HLA-matched HSCT, particularly in younger patients with R/R NHL without appropriate donors.

OUTCOMES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH ACUTE MYELOID LEUKEMIA HARBOURING INV(3)(q21;q26.2)/t(3;3)(q21;q26.2)

E1522

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Background: Acute myeloid leukemia (AML) with inv(3)(q21;q26.2)/t(3;3)(q21;q26.2) is one of the common cytogenetic abnormalities in AML, and is characterized by resistance to chemotherapy and poor outcomes. Therefore, the presence of this chromosomal abnormality in AML is an indication for allogeneic hematopoietic stem cell transplantation (allo-HSCT). However, outcomes of AML with inv(3)(q21;q26.2) remain unclear.

Aims: We retrospectively examined the impact of inv(3)(q21;q26.2) on the outcomes of allo-HSCT in patients with AML.

Methods: Clinical data were collected from the registry database of the Japan Society for Hematopoietic Cell Transplantation. We selected patients with AML harboring inv(3)(q21;q26.2), who were aged ≥16 years and underwent their first transplantation between January 2000 and December 2014. We analyzed outcomes such as overall survival (OS), disease-free survival (DFS), relapse and nonrelapse mortality (NRM) for the patients underwent allo-HSCT. OS was estimated using the Kaplan-Meier method and compared using the log-rank test. Relapse and NRM were considered as competing risk and were compared using the Gray’s test. In a multivariate analysis, the Cox proportional hazard model was used to analyze OS. The following variables were collected: sex, disease status at allo-HSCT, age taken for allo-HSCT from diagnosis, donor source, conditioning regimen, additional monosomy of chromosome 7 or partial deletion of long arm of chromosome 7 and type of 3q abnormality.

Results: Of 15025 patients with AML who were aged ≥16 years and who underwent first transplantation, inv(3)(q21;q26.2)(3;3)(q21;q26.2) was identified in 66 patients. The median age of patients was 46 years (range, 16-72 years). Of the 66 patients, 10 (15.2%) were in first complete remission (CR1) at allo-HSCT, 54 (81.8%) were in non-CR, and the disease status of two patients was unknown. The probabilities of 2-year OS, relapse, and NRM were 27.8% (95% CI 14.2%, 41.4%, 47.6-75.0), 21.1% (95% CI 11.8-32.3), respectively. Multivariate analysis revealed that an age of ≥50 years (HR, 2.05; 95% CI, 1.06-3.99; P=0.03) was significant risk factors for poor OS. Non-CR at transplantation (HR, 2.55; 95% CI, 0.94-6.93; P=0.07), and reduced conditioning intensity
Summary/Conclusions: These findings revealed that AML with inv(3)(t;3;3) had dismal outcome even after allo-HSCT. Multivariate analysis suggested that a myeloablative conditioning regimen might improve the transplantation outcome.

E1523

PHARMACOKINETICS (PK) OF PROPYLENE GLYCOL-FREE MELPHALAN HCL (PG-FREE MEL) IN MULTIPLE MYELOMA (MM) PATIENTS UNDERGOING AUTOLOGOUS TRANSPLANTATION (AHCT)

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Background: Melphalan (MEL) is the most commonly used conditioning agent in AHCT for MM and exhibits a dose response relationship (Nathi CE Br J Clin Pharmacol. 2010 May; 69(5):484). PG-free MEL (Evomela®) has longer stability in solution, results in a slightly higher systemic exposure compared with standard MEL and eliminates propylene glycol administration during high dose melphalan-based conditioning. This agent was shown to be bioequivalent to conventional melphalan leading to successful myeloablation and engraftment in MM pts receiving AHCT with no transplant related mortality or unexpected toxicity leading to its FDA approval (Hari P Biol Blood Marrow Transplant. 2015 Dec; 21(12):2100). Published studies thus far have used PG-free MEL in 2 consecutive daily doses of 100mg/m²/day while a single daily conditioning dose of 200mg/m² (MEL200) is most commonly used in clinical practice.

Aims: Determine the safety and PK variability of high dose PG-free MEL 200mg/m² in patients undergoing AHCT for MM

Methods: Open-label phase II study in which 10 serial blood samples at specific time points for the PK evaluation of melphalan were collected immediately prior to and after receiving single 200mg/m² dose of PG-free MEL on day -2 as a 2mg/ml solution. The primary objective was a descriptive analysis of melphalan PK while secondary objectives included the response rates, engraftment and the toxicity and safety profile of PG-free MEL conditioning.

Results: As of Feb 2017, a total of 24 pts. were enrolled (63% male) with a median age of 67 years (range 46-72), including 23 (96%) who received upfront AHCT and 1 (4%) after relapse (Figure 1). High-risk cytogenetics was present in 6 (25%) pts 25% were in ISS stage 3. Disease status at transplant was complete remission (CR) in 4 (17%), very good partial remission (VGPR) in 12 (50%) and PR in 8 (33%). AHCT was performed entirely as outpatient in 25%.

PK data are available for the first 12 pts at this time. Wide variability in MEL exposure was noted with maximum plasma concentration (Cmax) of 10,100 ng/ml, median Cmax 7750ng/ml (range, 5220-10,100) and median area under the concentration- time curve (AUC) of 561500 ng.min/ml (range, 771000-254000). Mean AUC was 549000 (±155000). No grade 4 non-hematologic toxicities or gastrointestinal toxicities were observed including in patients with Cmax >10,000 (upper quartile of distribution) or AUC>625000. All patients are alive and post-transplant responses in those with at least 100 days of follow up indicate sCR/CR in 60% and VGPR in 30%.

Figure 1.

Summary/Conclusions: PG-Free MEL can be safely administered as a single 200mg/m² dose in conditioning with a favorable toxicity profile. Considerable variability in the PK parameters of high dose MEL indicate that PK directed MEL dosing could be used to optimize MEL exposure. The safety profile of PG-free MEL indicates no increase in mucosal toxicity or adverse events seen even in subjects with highest levels of MEL exposure. For patients in the lowest quartile of AUC, increased PG-free MEL doses up to 20 to 40% over 200mg/m² may be safely attempted without additional toxicity if PK directed dosing is used to ensure adequate MEL exposure and utilize the dose response effect of MEL.

E1524

IMPAIRED LYMPHOCYTE RECONSTITUTION AFTER AUTOLOGOUS TRANSPLANT IS ASSOCIATED WITH APOPTOSIS OF CD8+ T CELLS AND PREDICTS ADVERSE CLINICAL OUTCOME


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Background: In patients undergoing autologous stem cell transplantation (ASCT), faster recovery of the lymphocyte counts has been associated with longer disease-free survival (DFS) and longer overall survival (OS). We noticed that the post-transplant lymphocyte counts fluctuated significantly during the first post-transplant weeks and wondered what the clinical significance of this observation is, and what dictates the lymphocyte counts over time.

Aims: Describe the kinetics of post-ASCT lymphocyte reconstitution in a single patient and across patients. Determine whether activation of anti-apoptotic pathways is associated with faster recovery of the lymphocyte counts.

Methods: We reviewed the medical records of 105 consecutive patients with lymphoma (Non-Hodgkin’s lymphoma and Hodgkin disease) or multiple myeloma who underwent ASCT at Tel-Aviv Sourasky Medical Center and were alive 24 weeks after the transplant. In each patient we documented the absolute lymphocyte counts (ALC) starting 2 weeks after the transplant until the 24th post-transplant week. We used flow cytometry to characterize the lymphocyte sub-populations in lymphocytes derived from 20 randomly selected patients, assayed apoptosis by DiO6 binding and used fluorescence anti-MO2 monoclonal antibody to detect the M02 epitope by flow cytometry. The probability of OS and of DFS was estimated by the Kaplan-Meier method. The log-rank test was used to compare survival distributions.

Results: The ALC was recorded at least once-weekly between the 2nd and 24th post-transplant weeks for each of the 105 study participants. The median ALC during the first 2-16 weeks was 1.4 X10³/μL (range: 0.3 to 4.1) and varied considerably in a single patient. After the 16th week, the ALC stabilized and divided the cohort into those with high (n=54, median =1.9 x10³/μL, range: 1.3 to 3.1) and low (n=51, median=0.9 x10³/μL, range 0.15 to 1.25) ALCs. Patients with low ALCs were slightly younger, but in all other patient or disease characteristics there were no differences between the two groups. Remarkably, the CD4+ subpopulation was low across all patients, and the difference in ALCs was primarily in the CD8+ subpopulation which remained low in half of the patients and normal or above normal in others. Interestingly, patients with prolonged lymphopenia had higher rates of apoptosis in freshly obtained lymphocytes and the expression levels of MO2, a CD14-derived epitope that protects the cells from apoptosis correlated with lymphocyte counts. Patients with high ALCs during 16-24 post-transplant weeks had longer DFS (P=0.07) and OS (P=0.04) compared to patients with low ALCs. In a multivariable analysis low ALC at 16 to 24 post-transplant weeks was the strongest predictor for shorter OS.

Summary/Conclusions: The analysis of post-ASCT lymphocyte counts revealed a unique pattern. It fluctuates during the first 4 post-transplant months and stabilizes thereafter, dichotomizing the patients into two groups. In all patients the CD4+ subpopulation remained low for at least 6 post-transplant months. However, in a subset of patients upregulation of intracellular anti-apoptotic signals was associated with recovery of the CD8+ subpopulation. In the
remaining, both CD4+ and CD8+ subpopulations remained low and these patients were prone to develop relapse. These findings underscore a putative function of CD8+ T-cells in eliminating post-transplant residual disease and maintaining the patients disease free.

E1525
COMPARISON OF TECAM AND BEAM HIGH-DOSE CHEMOTHERAPY FOLLOWED BY AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN LYMPHOMA: EFFICACY AND TOXICITY
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Background: High-dose chemotherapy conditioning regimens followed by autologous hematopoietic stem cell transplantation (AH SCT) generally provide good results in relapsed and refractory lymphomas.

Aims: Limited data are available to guide the choice of conditioning regimen before AH SCT for patients with lymphoma. We evaluated the efficacy and safety of TECAM and BEAM conditioning as autologous stem cell support in patients with relapsed/refractory lymphomas.

Methods: From July 2011 to October 2016, 64 pathologically confirmed lymphoma patients underwent AH SCT with BEAM (n=32) or TECAM (n=32) regimens in Hematology Division of Ege University Faculty of Medicine. Patients considered as high risk at diagnosis or with relapsed or refractory diseases were eligible for AH SCT. The two groups were well matched in terms of age, gender, histology. Patients were conditioned with TECAM (thiotepa [40mg/m² x four days], etoposide [200mg/m² x four days], cyclophosphamide [60mg/kg x one day], cytarabine [200mg/m² x four days] and melphalan [80mg/m² x two days]) or BEAM (carmustine [300mg/m² x one day], etoposide [200mg/m² x four days], cytarabine [200mg/m² x four days] and melphalan [140mg/m² x one day]) regimens.

Results: The estimated 22-months overall survival for the TECAM and BEAM groups were 53% and 63%, respectively (p=0.41). The estimated 22-months progression-free survival in the BEAM group (59%) was relatively inferior to the TECAM (74%) group, but the differences were not significant (p=0.98). Cardiotoxicities were relatively more common in the BEAM group. No differences were observed in the time to hematopoietic recovery, the duration of hospitalization, hematological and nonhematological toxicities.

Summary/Conclusions: We conducted a single-center retrospective on lymphoma patients undergoing AH SCT, comparing efficacy and toxicity of TECAM and BEAM conditioning regimens. These two regimens are all optional high-dose chemotherapy with favorable efficacy and acceptable toxicity.

E1526
GENETIC MARKERS OF THE NEUTROPENIA DURATION AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH MULTIPLE MYELOMA
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Background: The successes achieved in the treatment of multiple myeloma (MM) in the past few years, associated with the use of high-dose chemotherapy, and with the use of new drugs. Using high-regimes with subsequent autologous hematopoietic stem cells (auto-H SCT) has increased both overall and progression-free survival of patients with MM, as well as improved quality of life. In most cases, patients in the early post-transplant period have severe toxic and infectious complications of varying severity that requires resource-intensive supportive care. The duration of the period of hematopoiesis hypoplasia is dependent on many factors, and an average of 14-16 days. In turn, the attachment of infectious complications in some cases adversely affect the duration of neutropenia.

Aims: To evaluate the possible association of the immune response genes mutation status to the duration of neutropenia after autologous transplantation of peripheral blood stem cells in patients with multiple myeloma.

Methods: The study included 19 patients with multiple myeloma at the age of 32 to 67 years (median - 52 years) who underwent autologous transplantation of hematopoietic stem cells after conditioning regimen with high-dose melphalan. Among surveyed: 8 men and 11 women. In accordance with staging for Durie-Salmon (DSS) system in patients following stages of MM were installed: stage 1A in one patient (5.2%), stage 2A - in 12 patients (63.2%), stage 2B - in two patients (10.5%) and stage 3A - in four patients (21.1%). In the post-transplantation period, partial remission of the disease was achieved in seven patients (36.8%), very good partial remission - in eight patients (42.1%) and complete response in four patients (21.1%). Genotyping of polymorphisms of the innate immune response genes TLR2 (rs5743708), TLR3 (rs3775291), TLR6 (rs5743810), TLR9 (rs5743836), IL1β (rs2069764), IL2 (rs2069762), IL4 (rs2423250), IL6 (rs1800795), IL10 (rs1800871), IL17A (rs2275913), CD14 (rs34424920), TNFa (rs1800629), FCGR2A (rs1801274) was performed by polymerase chain reaction with allele-specific primers (Lifeh, Russia) at the time of diagnosis.

Results: Depending on the duration of the neutropenia period all examined are divided into two groups. The first group included 10 patients with MM who have early observed recovery (within the first 13 days, 11-13 days), the number of leukocytes ≥1000 cells per ml after auto-H SCT. The second group consisted of nine patients with agranulocytosis held more than two weeks (≥14 days, 14-19 days). When comparing the genotyping data found that a longer period of neutropenia after autologous HSCT was significantly associated with the presence in genotype of MM patients homozygous wild-type allele A gene IL17A at position -197 (OR 13.15, 95%CI: 0.60-288.34, p=0.03) and with a predominance of heterozygous mutant allele C of the gene IL1β at position -31 (OR 8.17, 95%CI: 1.03-67.94, p=0.04).

Summary/Conclusions: Our findings point to immune response genes involved in the rate of recovery of hematopoiesis in MM patients after autolo-
gous HSCT. Identification of the wild-type allele in intron gene IL17A (G-197TA) and mutant allele in intron gene IL1β (T-31C) will predict the risk of prolonging the period of agranulocytosis and, consequently, the risk of post-transplant complications, and develop a personalized strategy of managing them.

E1527
SUCCESSFUL TREATMENT WITH GRANULOCYTE TRANSFUSION AND EARLY NEUTROPHIL ENGRAFTMENT IN ALLOGENIC TRANSPLANT PATIENTS WITH FEBRILE NEUTROPENIA
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Background: Febrile Neutropenia is very severe and urgent early complication after bone marrow transplantation before engraftment. Infection delays engraftments. In this study we retrospectively evaluated the effect and outcome of Granulocyte transfusion on febrile neutropenia and neutrophil engraftment in patients receiving allogeneic transplantation.

Aims: Between 2015-2016, five patients receiving allogeneic bone marrow transplantation (BMT) were treated with granulocyte transfusion at the time of febrile neutropenia before engraftment. The reasons for the use of the granulocyte transfusion were prolonged febrile neutropenia episode.

Figure 1.

Methods: Five AML patients underwent allogeneic transplantation. Three of them transplanted from match sibling donors, one from unrelated donor, and one from (7/10) mismatch mother (haploidentic transplant). They had febrile neutropenia after transplantation, before engraftment. They were given antibi-
tics. Before the granulocyte transfusion, on the 13th-18th days of transplantation, their neutrophil counts were 0.03-0.08x10^3/dl.

Results: We started Granulocyte transfusion for three days. Granulocyte was collected from unrelated and same blood groups donors. Mean infused gran-
ulocyte counts were 3.6x10^10 (1.3-4.6x10^10)/day. Twenty-four hours after granulocyte transfusion, mean neutrophil counts were 0.6x10^3/dl (0.4-0.8x10^3/dl). Neutrophil counts were 2.6 x 10^3/dl (1.7-2.6x10^3/dl), after 48 hour. After 72 hours, neutrophil counts were 3.4x10^3/dl (2.1-4.5x10^3/dl). After 4th days of granulocyte transfusion, neutrophil counts were normal levels (>0.5x10^3/dl).  
Summary/Conclusions: Granulocyte transfusions during the febrile neutropenia, helped to better overcome febrile neutropenia periods in allogeneic transplant patients before engraftment. In addition, granulocytes transfusion also may help early neutrophil engraftments.

Results: A total of 229 patients were identified (MA-n=35, 15%; RIC-n=194, 85%). Acute myeloid leukaemia was the most common indication (n=103, 45%). Mean age at ASCT was 51 years (18-72 years). Median follow up after ASCT was 2.19 years (range 9.6-6.6 years). Overall survival to 100 and 365 days was 93% and 74% respectively. Pre-existing renal impairment was uncommon (mean eGFR 92ml/min, range 45-143ml/min). During the first 100 days, no differences were seen in mean eGFR in survival vs non-survival groups (75 and 80ml/min respectively, p=0.23). Amongst all patients, AKI incidence in the first 100 days was greater in the non-survival group (93.2% vs 80.6%, p=0.02). On multivariate analysis, AKI event in the first 100 days and HLA mismatch (<8/8) were independent factors predicting mortality (p=0.02 and p=0.04 respectively). Recipient age and gender, ASCT indication, history of hypertension, CMV status, donor sex, stem cell source and conditioning regimen (MA vs RIC) were not statistically significant (p>0.05). Within the first year of ASCT, pre-terminal AKI was noted in 29% (n=23) of all patients dying (n=59) with sepsis accounting for 44% of deaths. For non-relapse causes (n=57, 97%), only 11 (18%) had chronic renal impairment. Chronic GvHD was associated with these patients (73%) one of whom was dialysis dependent.

Summary/Conclusions: AKI is a very common post ASCT. Chronic renal failure is uncommon in long-term survivors. AKI is however a prominent event preceding death. Consistent with other reports AKI and HLA mismatch correlated inferior outcomes. Poor survival from AKI probably reflects physiological strain from other complications (e.g. sepsis and GvHD). Early recognition and treatment of AKI are important measures in the supportive care of patients with AKI.

E1528

DEFIBRITODE FOR THE PREVENTION AND TREATMENT OF HEPATIC VENO-OCCLUSIVE DISEASE AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION; A SINGLE CENTER EXPERIENCE

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Background: Hepatic veno-occlusive disease (VOD) is a common and serious complication of hematopoietic stem cell transplantation (HSCT) in children. We aimed to assess prospectively the use of prophylactic defibrotide in pediatric patients undergoing HSCT.

Aims: We aimed to assess prospectively the use of prophylactic defibrotide in pediatric patients undergoing HSCT.

Methods: In this study, 113 patients who underwent HSCT were given defibrotide prophylaxis as 25mg/kg per day in four divided intravenous infusions over 24 hours, starting on the same day as the pretransplantation conditioning regimen. The mean duration of use of defibrotide is 25 days as a prophylaxis.

Results: In this study, 113 patients were recruited, 66 male patients and 47 female patients, with the average of 9.1 years, range 1-20; 8% infants, 55% children and 37% adolescent. There were 50 patients with thalassemia major, 41 patients with leukemia, 11 patients with aplastic anemia, one patient with Diamond Blackfan anemia, two patients with congenital dyserythropoietic anemia, one patient with osteopetrosis, four patients with familial hemophagocytic lymphohistiocytosis, two patients with severe immune deficiency and one patient with Kostman syndrome. All transplants were allogeneic. No serious side effects were observed during patients development of clinical VOD (Seagate criteria). In these patients, defibrotide dose was increased to a treatment dose of 40-60mg/kg per day. One infant patient with Kostman syndrome died due to hepatic and pulmonary veno-occlusive disease. After 36 months of follow up, 7 patients who developed VOD are being well and no patient have transplant related complications.

Summary/Conclusions: Hepatic veno-occlusive disease, which is caused by hepatitis and sinusoidal vessel endothelium damage, may occur early after HSCT, and in its severe form, may lead to liver failure, hepatorenal syndrome, portal hypertension, and eventually death from multorgan failure. In this prospective study, we demonstrated that the use of defibrotide is safe and effective in preventing and treating VOD in pediatric patients at high risk.

E1529

ACUTE RENAL IMPAIRMENT IN ALLOGENEIC STEM CELL TRANSPLANT RECIPIENTS, A PREDICTOR OF MORTALITY

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Background: Allogeneic stem cell transplant (ASCT) remains the only curative option in many malignant and non-malignant conditions. There remains however, unmet needs of identifiable morbidity and mortality. One risk, acute kidney injury (AKI), can result from drug toxicity and/or haemodynamic instability from sepsis and/or graft vs host disease (GVHD). Existing reports on the impact of AKI have concentrated on patients undergoing mainly myeloablative (MA) conditioning alone, whilst those undergoing reduced intensity conditioning (RIC) transplants have reported outcomes from limited patient numbers.

Aims: To investigate the incidence, causes and consequences of AKI in patients undergoing ASCT, including survival.

Methods: The prospectively maintained database of the South Wales Blood and Marrow Transplant programme which serves 77% of the Welsh population, was interrogated to identify patients undergoing ASCT from January 2010 to December 2015. Patients received ciclosporin as GVHD prophylaxis to 100 days post ASCT and weaned thereafter in the absence of GVHD. Serum creatinine and derived estimated glomerular filtration rate (eGFR) acted as the main assessment of renal function. The Acute Kidney Injury Network classification was used to grade AKI. Causes of AKI were assigned after independent review of clinical notes and relevant laboratory data. Patients undergoing second ASCT were excluded. Statistical analysis was carried out using SPSS, version 23 including COX regression and Kaplan-Meier survival analysis.

Results: A total of 368 patients were identified, 66 male patients and 47 female patients (73%) one of whom was dialysis dependent. AKI is however a prominent event preceeding death. Consistent with other reports AKI and HLA mismatch correlated inferior outcomes. Poor survival from AKI probably reflects physiological strain from other complications (e.g. sepsis and GvHD). Early recognition and treatment of AKI are important measures in the supportive care of patients with AKI.
in the low, intermediate, high and very high risk groups, respectively, showing a clear distinction by categories (p < 0.038) (figure 1). Refraining re-relapse, 44.28% (6.6%) of patients relapsed. Neither PAM nor HCT-CI were good predictors for relapse. However, HCT-CI was not good predicting complications, GVHD, NRM or relapse.

Summary/Conclusions: In our series of pts, risk-groups based on PAM score provided much better discrimination of post-HSCT complications, aGVHD (II-IV) and NRM than HCT-CI model. None of the indexes were acceptable predictors of relapse. Furthermore, correlation between both indexes was poor.

E1531

ROLE AND TIMING OF HEMATOPOIETIC CELL TRANSPLANTATION FOR HIGH-RISK PERIPHERAL T-CELL LYMPHOMAS

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222nd Congress of the European Hematology Association

Background: Peripheral T-cell lymphomas (PTCLs) often carry poor outcomes with conventional chemotherapy, and hematopoietic cell transplantation (HCT) can benefit patients with PTCL. Recent retrospective studies have reported that autoHCT as consolidation can offer a durable survival benefit in high-risk patients with first complete or partial response, and alloHCT could result in long-term disease control for relapsed and refractory patients.

Aims: To explore questions about the optimal timing for stem cell transplantation and relative efficacy of auto-HCT versus alloHCT.

Methods: We conducted a retrospective review of 67 patients with peripheral T-cell lymphoma who underwent autologous HCT (autoHCT, n=43, median age 40 years) or allelogeneic HCT (alloHCT, n=24, median age 36.5 years) from 2004 to 2016.

Results: With a median follow-up of 27 months, 5-year PFS and OS of auto-HCT patients were 49% and 57%, respectively. Among alloHCT recipients, the 5-year PFS and OS were 54% and 55%, respectively. When considering incidence of disease relapse or progression (PFS) and nonrelapse mortality (NRM), the 5-year CI and 1-year NRM of alloHCT recipients were 38% and 18%, respectively, and 58% and 7% of autoHCT patients, respectively. There were no differences between autoHCT and alloHCT on 5-year PFS (P=0.499), OS (P=0.566), CI (P=0.555) and NRM (P=0.202). When specifically examining recipients in primary refractory disease, 3-year PFS rates of autoHCT and alloHCT were 20% and 49% (P=0.054), 3-year OS rates were 20% and 53% (P=0.042), respectively.

Figure 1.

Summary/Conclusions: This analysis shows that HCT can benefit patients with high-risk PTCL in both remission and primary refractory setting. The outcomes did not differ significantly between autoHCT and alloHCT approaches, but alloHCT recipients in primary refractory disease resulted in significantly better outcomes than autoHCT patients. So, we favor proceeding to alloHCT if patients with PTCL in primary refractory disease.

E1532

IMPACT OF BASELINE BILIRUBIN ON SURVIVAL IN PATIENTS WITH HEPATIC VENO-OCCCLUSIVE DISEASE/SINUSOIDAL OBSTRUCTION SYNDROME RECEIVING DEBIFROBIDATE: POST-HC ANALYSIS OF EXPANDED-ACCESS PROTOCOL FINAL DATA

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Background: Veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS) is an unpredictable, potentially life-threatening complication of hematopoietic stem cell transplantation (HSCT) conditioning. VOD/SOS with multi-organ dysfunction (MOD) may be associated with ≥80% mortality. Defibrotide is approved in the European Union to treat severe hepatic VOD/SOS post-HSCT and in the United States to treat hepatic VOD/SOS with renal or pulmonary dysfunction post-HSCT. Prior to approval, defibrotide had been available in the United States via an expanded-access program.

Aims: A post-hoc analysis of final data from the defibrotide expanded-access program was used to explore Day +100 survival post-HSCT based on bilirubin-level categories at the time of study entry.

Methods: Patients in the defibrotide expanded-access program had VOD/SOS diagnosed by investigators using Baltimore criteria (bilirubin ≥2mg/dL and ≥2 of: hepatomegaly, ascites, ≥5% weight gain), modified Seattle criteria (≥2 of: bilirubin ≥2mg/dL, hepatomegaly, or ascites and/or ≥5% weight gain), or biopsy; bilirubin ≥2 was not required for modified Seattle criteria or biopsy. MOD (moderate to severe hepatic, pulmonary + MOD) was defined as treatment (25mg/kg/day) was recommended for ≥21 days. Here, Day +100 survival was explored by bilirubin level at study entry using categories that are part of the European Society for Blood and Marrow Transplantation (EBMT) proposed grading scale for adults (≥2 to <3mg/dL, ≥3 to <5, ≥5 to <8, and ≥8), as well as bilirubin ≥2mg/dL, which is not part of the scale but has been reported in children with VOD/SOS.

Results: There were 1000 HSCT patients enrolled, between December 2007 and September 2016, with a confirmed diagnosis of VOD/SOS and receiving ≥1 dose of defibrotide, 512 patients had MOD. Median age was lowest in patients with bilirubin <2 (4.6 years); 19% of patients), Median age was 16 years in the bilirubin ≥2 to <3 group (53.5% of patients) and 13.5 in the ≥3 to <5 group (20.4% of patients); median age in other groups ranged from 15 to 17 years. Kaplan-Meier estimated Day +100 survival in all HSCT patients was 58.9%, with 85.6% in patients with BR ≤2; other bilirubin groups were older and survival estimates decreased (Table 1). In the pediatric (aged ≤16 years) and adult (aged >16 years) patients, patterns were similar (Table 1). Estimated survival rates were lower for patients with MOD across all groups. Of all 1000 HSCT patients with confirmed VOD/SOS, 210 (21%) had treatment-related AEs (TRAEs). The TRAEs in ≥2% of patients were pulmonary hemorrhage (4.6%), gastrointestinal hemorrhage (3.0%), epistaxis (2.3%), and hypotension (2.0%).

Table 1. Day +100 Survival (Kaplan-Meier, N=1000).

<table>
<thead>
<tr>
<th>Bilirubin (mg/dL)</th>
<th>HSCT Patients</th>
<th>Age ≤16 Years</th>
<th>Age &gt;16 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2</td>
<td>97</td>
<td>85.0%</td>
<td>133.0%</td>
</tr>
<tr>
<td>≥2 to &lt;3</td>
<td>555</td>
<td>55.5%</td>
<td>378.63%</td>
</tr>
<tr>
<td>≥3 to &lt;5</td>
<td>264</td>
<td>47.2%</td>
<td>120.56%</td>
</tr>
<tr>
<td>≥5 to &lt;8</td>
<td>39</td>
<td>53.7%</td>
<td>22.54%</td>
</tr>
<tr>
<td>≥8</td>
<td>30</td>
<td>39.3%</td>
<td>11.13%</td>
</tr>
</tbody>
</table>

Summary/Conclusions: This post-hoc analysis found that higher bilirubin levels were generally associated with lower Day +100 survival. These results should be interpreted with caution, as only 1 EBMT criterion was analyzed. MOD was also associated with lower Day +100 survival. The results suggest that diagnosis and treatment of VOD/SOS, before bilirubin becomes markedly elevated, may be associated with improved outcome.

Support: Jazz Pharmaceuticals.

E1533

LONG-TERM FOLLOW-UP OF A PROSPECTIVE TRIAL OF INTENSIFIED CHEMO-IMMUNOTHERAPY WITH AUTOLOGOUS OR ALLOGENEIC STEM CELL TRANSPLANTATION IN PATIENTS AFFECTED BY PERIPHERAL T-CELL LYMPHOMA

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Background: Peripheral T-cell lymphoma is a rare entity with a poor outcome in terms of survival. HCT is considered as a treatment giving a significant survival benefit in selected patients.

Aims: To assess long-term survival of patients affected by PTCL, who were enrolled in a prospective trial comparing intensified chemotherapy with autologous or allogeneic HSCT.

Methods: Between 1993 and 1995, 77 patients were enrolled in a prospective trial comparing intensified chemotherapy with autologous or allogeneic HSCT. The following management arm was used: (1) intensified chemotherapy with autoHSCT, (2) intensified chemotherapy with alloHSCT, and (3) intensified chemotherapy alone (control group). The survival analysis was performed using the Kaplan-Meier method.

Results: The median follow-up was 21 years. The survival rates at 10 years for the autoHSCT group were 40%, for the alloHSCT group were 25%, and for the control group were 15%. The differences were statistically significant (p<0.05).

Summary/Conclusions: The results of this study suggest that intensified chemotherapy with autologous or allogeneic HSCT may provide a significant survival benefit in patients affected by PTCL.

Support: Jazz Pharmaceuticals.
E1534

UNRELATED DONOR ATTRITION AT A LATE STAGE: THE BRITISH BONE MARROW REGISTRY EXPERIENCE

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Background: The success of searches for unrelated stem cell donors (UDs) relies on the existence of large international donor registries and the availability and reliability of donors on the register. Donor attrition at the verification typing (VT) or later stage results in delay of transplant and can adversely affect patient outcomes. The British Bone Marrow Registry (BMMR) provides UDs to international centres (TcSs) and to UK TcS via an international network. Data reported by international registries on donor attrition is scarce and mainly focused on attrition at the VT stage. BMMR donors are recruited from blood donors and may differ in their reliability from non-blood donors included in existing reports.

Aims: To investigate donor attrition rates and causes of cancellation among finally selected or backup BMMR donors at the post-VT stage.

Methods: Data on requests for work-ups from April 2002 to December 2016 were extracted from BMMR databases and donor notes and were analysed retrospectively. The reasons for cancellation were categorised: cancellation initiated by the donor (in rare mixed reasons. Within donor reasons we distinguished 3 categories: donor medical reasons, donor withdrawal on personal grounds and inability to contact the donor. We examined associations between cancellations for donor-related reasons and the following factors: donor sex, age at time of donation/cancellation, time on the register and donor reliability score. The reliability score relates to blood donation and runs from 1 (best) to 5 (worst), increasing if a donor fails to attend appointments for blood donation.

Results: A BMMR final/breakdown donor was selected for 3184 stem cell or lymphocyte collections. 82% of the requests (n=2613) were completed. Out of the 571 (18%) cancelled cases the reason for cancellation was not available for 5 cases. Overall, more than half of the cancellations (n=302, 53%) were prevented by TcS mainly due to patient death, deterioration or alternative donor choice. Donor reasons accounted for 38% of cancellations (n=216, 6.8% of requested donors), of which 69% (n=148) happened for medical reasons, 27% (n=59) for donor pull-out on personal grounds and 4% (n=9) due to uncontactable donors. The medical reasons for withdrawal were varied but the most frequent health issues were obesity and cardiovascular disease. Analyses of factors affecting donor reasons showed that donor sex and time on the register were not associated with donor fitness or withdrawal rate. Age had no impact on donor pull-out, but it was significantly associated with medical eligibility and donors who were male were more frequently medically eligible. Donor pull-out was associated with blood donor reliability score (p=0.029, score 5 vs others). In 48 cases (8%) there were mixed reasons where TcS had other donor options and pursued them because of issues such as donor availability for ideal dates or CMV mismatches.

Summary/Conclusions: In our registry patient-related issues accounted for more than half of cancellations at a late stage in the stem cell donor pathway. Cancellations for donor reasons were unusual (6.8% of requested donors), which figure compares favourably with international data (12.4% of requested donors, WMDA Annual Report 2015). This is likely due to the fact that most BMMR donors are regular blood donors: few donors withdrew for personal reasons (6.8% of requested donors). Further work is underway to allow earlier or reduced deferral of medically unsuitable donors such as control of high blood pressure and to explore personal reasons which cause donors to withdraw. This study should provide reassurance to TcS that BMMR donor are reliable and accessible stem cell donors.

E1535

POLIMORPHISM IN TGFβ1 GENE PREDISPOSES TO RELAPSE AND DEVELOPMENT OF ACUTE GRAFT-VS-HOST DISEASE GRADES III-IV

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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the most effective treatment option for certain hematologic malignancies. Cytokines play a well established role in the mechanism of acute GvHD (aGvHD), which is one of the most significant complications of allo-HSCT. The transforming growth factor B1 (TGFβ1) gene, encoding aGvHD. The success of searches for unrelated stem cell donors (UDs) relies on the existence of large international donor registries and the availability and reliability of donors on the register. Donor attrition at the verification typing (VT) or later stage results in delay of transplant and can adversely affect patient outcomes. The British Bone Marrow Registry (BMMR) provides UDs to international centres (TcSs) and to UK TcS via an international network. Data reported by international registries on donor attrition is scarce and mainly focused on attrition at the VT stage. BMMR donors are recruited from blood donors and may differ in their reliability from non-blood donors included in existing reports.

Aims: To investigate the role of TGFβ1 -1347C>T polymorphism in the outcome of HSCT.

Methods: We examined the association of recipient and donor TGFβ1 -1347C>T and allo-HSCT outcome in a cohort of 419 adult patients who underwent first allo-HSCT between January 2007 and December 2013 at our single center. 217 patients received stem cells from their siblings, 202 patients from matched unrelated donors (MUD). For identification of TGFβ1 rs1800496 from genomic DNA LightCycler melting curve analysis (LightCycler 480II, Roche Diagnostics) was performed.

Results: We did not find any association between recipients' TGFβ1 -1347C>T polymorphism and HSCT outcome. However, in patients whose unrelated donors carried homozygous TGFβ1 -1347TT variant, aGvHD grades III-IV occurred more frequently (aGvHD grade III-IV: 28.9% vs aGvHD grade 0-1: 9.6%, p=0.006). Similar finding was observed on a subgroup of patients with acute leukemia: in aGvHD grade III-IV 37.5%, while in grade 0-1 11.5% of patients had TT genotype (p=0.022). Donor TT genotype did not influence the relapse rate significantly. Patients with MUD carrying TT genotype had lower overall survival (OS) that of donors bearing at least one G variant, but the difference was not reached statistical significance (OS at 40 month for CC and CT variant donors: 45.3% and for TT donors: 26.2%). In case of sibling donors, we did not find association between recipient or donor genotype and aGvHD, but relapse rate was increased if donor had at least one T variant (n=115, 67.9% vs 32.1%, p=0.028). Significant differences in OS between the subgroups with different genotypes were not observed.

Summary/Conclusions: Our findings suggest that TGFβ1 -1347C>T polymorphism in HSCT donors might influence the development of aGvHD in unrelated and the relapse rate in related HSCT.

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E1536
EARLY AND LATE LOSS OF PROTECTIVE ANTIBODY LEVELS AGAINST MEASLES, MUMPS AND RUBELLA IN PATIENTS GIVEN ALLOGENIC HEMATOPOIETIC CELL TRANSPLANTATION
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Background: Live-vaccines should be avoided in the early period following allogeneic hematopoietic cell transplantation (HCT), due to a possible uncontrollable proliferation of the attenuated strains. The post HCT immune system is severely compromised by pharmacological immunosuppression and disruption of lymphoid tissues by conditioning and donor T cell alloreactivity. Patients frequently lost their antibody-based immunity against measles, mumps, and rubella after receiving allogeneic HCT.

Aims: Here, we studied the dynamics of antibody (AB) titers against measles, mumps, and rubella post-HCT.

Methods: We retrospectively analyzed serial AB titers in 240 patients who underwent allogeneic HCT from related or unrelated HLA-matched donors from 2002-2014 at our center. AB titers against measles, mumps and rubella were measured prior to HCT, at 6 months (m), and every year (y) post-HCT.

Results: Most patients had protective AB titers (measles 90%, mumps 86%, rubella 92%) prior to HCT. AB protection against mumps was lost in a substantial proportion of patients after HCT (protective AB titers in 72%@1y, 56%@5y, 50%@8y), comparing to AB against measles, which persist more frequently (protective AB titers in 85%@1y, 74%@5y, 73%@8y). We found a faster lost of protective AB in the first years for patients given a myeloablative condition (MAC) in comparison to patients with reduced condition (RIC), but the proportion of seropositive patients became more equal over time (Figure 1 displays the percentage of seropositive patients to Measles AB given MAC or RIC during 8 years post-HCT). The proportion of patients who retained protective AB titers at 5y post-HCT was higher in recipients of mobilized peripheral blood compared with bone marrow (BM) grafts (measles p=0.01, mumps=p=0.06, rubella=p=0.08).

For rubella, absolute AB titers were available. Patients with lymphoid malignancies, ongoing GVHD and pharmacological immunosuppression had a steeper decline of rubella AB titers as compared to patients with myeloid malignancies.

Figure 1.

Summary/Conclusions: We found a marked decline of AB titers post-HCT with loss of protection in a substantial proportion of patients. Surprisingly, BM grafts did not provide better AB protection post-HCT, despite their higher content of (donor) plasma cells. Together with the observations that (i) patients with lymphoid malignancies (who have received (B-) lymphocyte targeted therapies prior to HCT) had lower AB levels, while (ii) those given reduced intensity conditioning have a higher percentage of protective AB levels in the first years, our data suggest, that residual host plasma cells significantly contribute to AB production during the first years post-HCT. In opposite, the loss of protective AB levels in later years after transplantation was independent of the toxicity of the conditioning regime and may be a effect of weakening signaling for host plasma cells or late donor alloreactivity.

E1537
MICA AND NKG2D POLYMORPHISMS HAVE A SIGNIFICANT IMPACT ON GRAFT VERSUS HOST DISEASE AFTER HLA-MATCHED HEMATOPOIETIC STEM CELL TRANSPLANTATION.
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Background: MICA (MHC class I polypeptide-related sequence A) is a highly polymorphic gene closely linked to the HLA-B locus. It encodes a cell stress inducible glycoprotein, which mediates an activatory signal towards the NKG2D receptor expressed on NK-cells, CD8+ T-cells and NKT-cells. MICA polymorphisms have been shown to influence NKG2D signaling. Indeed, a methionine to valine change at position 129 in exon 3 categorized the MICA alleles into strong (MICA-129 met) and weak (MICA-129 val) binders of NKG2D receptor.

5 repetitions of GCT with 1 additional nucleotide insertion (G) in exon 5 designed the MICA A5.1 alleles with a premature stop codon. Moreover, NKG2D polymorphisms identified alleles associated with a low (NKC3 C/C and NKC4 C/C) or high cytotoxic activity (NKC3 G/G and NKC4 T/T).

Aims: In this study, we hypothesized that polymorphisms at the MICA and NKG2D loci are associated with adverse outcomes in HSCT.

Methods: Here, we evaluated whether recipient MICA and donor NKG2D polymorphisms (respectively MICA-129, MICA-1,1 and NKC3, NKC4) could influence the incidence of acute and chronic graft-versus-host disease (GVH), overall survival (OS) and relapse free survival (RFS) on 124 patients undergoing allogeneic hematopoietic stem cell transplantation using an HLA-matched donor (10/10).

Results: In an univariate model, recipient MICA A5.1 heterozygosity (p=0.030) and donor NKC4 C/C polymorphism (p=0.013) are associated with the increase of incidence of acute GVH (grade I to IV). Recipient MICA A5.1 heterozygosity is also associated with chronic GVH (p=0.04) while Recipient MICA-129 val/val tends to be a risk factor of chronic GVH without being statistically significant. These polymorphisms have no significant impact on OS and RFS in our study (median of follow up=15 months; range 0.2-49 months).

Summary/Conclusions: Our data suggest that a MICA or NKG2D low activity status can be related to an increase of acute GVH according to a mechanism that remains to be elucidated, maybe by a low cytotoxic activity on recipient dendritic cells.

E1538
STEM CELL TRANSPLANTATION WITH MYELOABLATIVE CONDITIONING USING TIMED SEQUENTIAL BUSULFAN IMPROVES OUTCOMES IN OLDER AML AND MDS PATIENTS
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1Stem Cell Transplantation and Cellular Therapy, The University of Texas MDACC, Houston, United States

Background: We previously reported 6% 100 day NRM with a MA fludarabine (Flu) and busulfan (Bu) in older patients with a median age of 60 years. MA dose of Bu in this timed sequential (TS) regimen was administered over a longer period of time. To assess its impact on survival, we compared the outcomes of older patients treated with the TS Bu (TS cohort) and the reduced intensity conditioning with Flu/Bu regimen, which is used as standard (ST) for older patients at our center ST cohort.

Aims: To assess its impact on survival, we compared the outcomes of older patients treated with the TS Bu (TS cohort) and the reduced intensity conditioning with Flu/Bu regimen, which is used as standard (RIC cohort) for older patients at our center.

Methods: Patients in the TS cohort received Flu 40mg/m²/day followed by IV Bu daily for 4 days (day -6 to -3) followed to achieve a total Bu course AUC of 20,000μmol-min based on PK studies. Patients in the ST cohort received Flu 40mg/m² day followed by IV Bu daily for 4 days (day -6 to -3) followed to achieve AUC of 16,000μmol-min. Patients with AML or MDS were eligible for the study if they had adequate organ function, had matched related or unrelated donor and were treated between Jan 2012 and Sep 2014.

Results: Patient characteristics including age, sex, disease status, cytogenetic risk group, donor type, graft source, CMV status and comorbidity were similarly distributed between the two cohorts. Median age was 66 and 65 years in TS-MAC and RIC cohorts respectively. Overall survival (OS) and progression free survival (PFS) were significantly better in the TS-MAC cohort. This was due to a reduction in the disease progression without any increase in the TRM. After adjusting for other covariates, the multivariate analysis for PFS confirmed the benefit of TS Bu regimen (HR: 0.36; P=0.003). The benefit was mainly seen in patients with a comorbidity score ≤3.

Table 1.
HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION WITH DEPLETION OF TCR αβ (+) IN CHILDREN: ERCIYES PEDIATRIC BMT CENTER

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Background: Recently, haploidentical hematopoietic stem cell transplantation (HSCT) posses an alternative option for patients without a suitable donor. Erciyes Pediatric BMT Center is the first pediatric center for haploidentical HSCT with depletion of TcR αβ (+) in Turkey.

Aims: We would like to share our pediatric experience with a follow up period of 5 years.

Methods: All children who underwent haploidentical HSCT in our center from December 2012 to February 2017 were included in the study. Total 51 haploidentical HSCT in 44 children (17 relapsed/refractory AML, 9 relapsed/refractory ALL, 4 SAA, 4 HLH, 2 Fanconi aplastic anemia, 2 Griscelli syndrome, 1 JMML, and 5 SCID) were performed. Transplantation-related mortality (TRM) was 13.7%. The regimen included ATG, Fludarabine, Thiotepa, Melphalan. Mycophenolate mofetil (MMF) was given as GvHD prophylaxis if the graft contained≤5 x10^6/kg TcR αβ (+).

Results: The mean of collected CD34 cells were 18.60 (range 3.98-43.66) x 10^6/kg. The graft had a purity of 99.9% TcRαβ depletion with a median of 0.257 (range 0.003 to 1.47) x 10^6 TcRαβ cells. The median engraftment days for myeloid and platelet were both 12th day of HSCT (range 7 to 28, 9 to 33 day) respectively. Grade II skin GvHD was detected in 8 patients, and treated with steroids without any further complications. However grade III, and grade IV gastrointestinal GvHD were observed in three patients. Although the patients with gastrointestinal GvHD were treated with steroid, bendosud, cyclosporine, MSC; one patient did not respond and died. MMF was given as GvHD prophylaxis in 36 patients and 15 patients did not receive any immune suppressive drug. The mean day of discharge was 34th day of HSCT. The long term follow up including immunological reconstructions were performed in 18 patients. The analysis of the immune reconstitution of the patients transplanted in haploidentical HSCT group showed a rapid immune reconstitution for CD3+ T cells 732 (range 126-2432)/mm3; for CD4+ helper T cells 92 (range 1-419)/mm3; CD8+ T cytotoxic cells 310 (range 95-2235)/mm3 at 28th day of HSCT. Ten brain stem patients are currently alive, with a median follow up of 22 months (range 1 to 49 months). Overall survival was 65.9% in these group.

Summary/Conclusions: Our primary results underline that haploidentical HSCT with depletion of TcR αβ (+) can be an option in experienced center in countries which unrelated donor programs are not satisfactory, as in Turkey. The availability of a haploidentical donor in most families is a potential advantage. Moreover probably more potent graft-versus tumor effect can be induced with haploidentical HSCT.

SECONDARY MYELODYSPLASTIC SYNDROME AND/OR ACUTE LEUKEMIA INCIDENCE AFTER AUTOLOGOUS TRANSPLANTATION FOR LYMPHOMA PATIENTS IS CONNECTED WITH DECREASE OF HEMATOPOIETIC RESERVE

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1Charles University General Hospital, 2Institute of Hematology and Blood Transfusion, Prague, Czech Republic

Background: Secondary myelodysplastic syndrome and acute myelogenous leukemia (sMDS/AML) is one of the most important long term complication of high dose therapy (HDS) with autologous stem cell transplantation (ASCT). The factors usually described to be associated with sMDS/AML development are pretreatment, HDS itself, radiotherapy, age and recently the evidence of TP53 mutations (Wong, Nature 2015) or clonal hematopoesis (Gibson, JCO 2016) is the only predictable factor for ASCT.

Aims: The aim of the study was to analyse the incidence and risk factors for sMDS/AML after HDT and ASCT for lymphoma.

Methods: Patients who underwent HDT with ASCT for lymphoma in one centre since 12/1993 till 7/2016 were analysed. Pretreatment characteristic, grad quality, engraftment characteristics were included into analysis. Patients were censored at the time of death or allogeneic stem cell transplant. Pearson, Kaplan Maier, log-rank and cox regression tests were used.

Results: Altogether 728 pts underwent ASCT for lymphoma in given time period. Out of which 19% consisted out of 77% B-NHL (568), 6% T-NHL (n 43) and 16% HL (n 119), 58% were men, age median at the transplant was 49 years (18-71). The median of previous lines was 2 (1-9). The stem cell collection was performed after chemotherapy and G-CSF mobilization in most cases, 19 pts were mobilized by G-CSF only and bone marrow only was used in 4 pts. The target CD34 dose was 3 x10^6/kg. The median number of apheresis was 2 (1-12). At the time of ASCT 90.6% of patients had chemosensitive disease (51.1% CR) and 9.4% were transplanted for chemoresistant disease. Tandem HDT and ASCT was used in 36 pts, BEAM was the most frequent HDT regimen (92.5%, 15 pts received ibritumomab tiuxetan and BEAM), the total body irradiation was used only in 4 pts, the rest of the patients received other chemother-apy regimens (CPB, thiopeta based, ICE and others). All pts except 4 received peripheral blood progenitor cells (PBPC) with median CD34 dose 8.6 x10^6/kg (0.4-115.5). BM was used in 22 cases (in 18 together with PBPC). G-CSF was administered from day +7. Involved or extended field radiotherapy either during pretreatment or in the period after ASCT was used in 37.7% of pts. With median follow-up 7.2 years there were observed 19 cases of sMDS/AML. The cumulative sMDS/AML incidence was at 5, 10 and 15 years 2.7%, 4.0% and 5.3% (figure A).

Figure 1.

Summary/Conclusions: The risk of sMDS/AML was 4.0% at 10y after ASCT and was connected with heavier pretreatment, which leads to the decrease of BM reserve, hematopoietic clonal development. The lower dose of CD34+ cell, the involvement to use BM progenitor cell and prolonged platelet engraftment could be considered as classical markers of these biological processes.

USE OF DEFIBROTIDE TO TREAT TRANSPLANT-ASSOCIATED THROMBOTIC MICROANGIOPATHY

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1Hospital Universitario Puerta de Hierro Majadahonda (Madrid), Majadahonda, Spain

Background: Transplant-associated thrombotic microangiopathy (TA-TMA) is a severe early transplant complication which results from endothelial injury and it exhibits characteristics of an atypical hemolytic uraemic syndrome. Beyond removal or treatment of precipitating factors and, more recently, treatment with eculizumab, TA-TMA remains a therapeutic challenge. Defibrotide, with marked protective effects on the endothelium and the potential to restore thrombocytopenia, may provide an alternative option for TA-TAM.

Aims: To analyze our center’s experience in the treatment of TA-TMA with defibrotide.

Methods: We reviewed all cases of TA-TMA treated with defibrotide in our allo- geneic transplant recipients between October 2008 and November 2016. All cases had non-immune hemolytic anemia with high LDH, low haptoglobin and negative Coombs test, >2 schistocytes per high-power field and thrombocytopenia (<50x10^9/L or <50% of normal baseline). Cases without signs of renal or neurological dysfunction were included (Deville, Transplantation 2014).

Results: We identified 17 TA-TMA episodes treated with defibrotide in 16 allo- geneic transplant recipients: 9 men; median age 38 years old (16-57); 10 sin- gle-cord blood plus third-party donor cells [Bautista G, 2009], 3 HLA-identical siblings and 3 unrelated donors; 13 myeloablative conditioning regimen, 10 with total body irradiation (Table 1). Co-morbid risk factors at the time of TA- TMA onset were: calcineurin inhibitor treatment in all cases (13 cyclosporin, 4 tacrolimus), acute GvHD grade III/IV in 8 cases, 3 CMV reactivations and 2 severe fungal (1 pulmonary aspergillosis, 1 Scedosporium Prolificans sep- ticemia) infections. Median onset of TA-TMA was on day +143 after transplant (2-536), 11 cases of early onset (<2 months) and 6 of late onset. Nine episodes were probable TA-TMA without organ dysfunction, 8 had renal failure and 2 presented with concomitant diffuse alveolar hemorrhage. First line replacement of calcineurin-inhibitors for basiliximab or other...
drugs for GVHD was performed in all cases. Defibrotide was subsequently administered as monotherapy in 5 cases, in combination with rituximab and/or plasma exchange in 7, and with other agents in 5 others (2 vincristine; 1 ecuclizumab; 1 bevacizumab; 1 mesenchymal stromal cells). Complete resolution of TA-TMA (CR) was achieved in 11 episodes (65%), and associated with a reduced all-cause mortality: 18% of CR cases (2/11: 1 multilobar pneumonia and 1 toxic encephalopathy) versus 83% of cases without CR (5/6, P=0.035; 1 TA-TAM-related diffuse alveolar hemorrhage, 1 lymphocytic encephalitis and 3 severe infections). TA-TMA cases triggered by severe infections had early (days 4, 11 and 15 after onset) and higher mortality rates (3/3, 100% vs 4/14, 29%; P=0.023). Rates of CR were higher in cases of probable TA-TAM without renal failure (8/9, 89% vs 3/8, 38%; P=0.027) and early-onset TA-TAM (9/11, 82% vs 2/6, 33%; P=0.046).

Table 1.

<table>
<thead>
<tr>
<th>TA-TMA episodes and per se to defibrotide</th>
<th>CR</th>
<th>Resolved</th>
<th>Partial</th>
<th>Died</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of episodes</td>
<td>11</td>
<td>9</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Age at CRs (years)</td>
<td>30.3</td>
<td>31.6</td>
<td>29.4</td>
<td>31.6</td>
</tr>
<tr>
<td>Sex</td>
<td>6 (55%)</td>
<td>3 (27%)</td>
<td>2 (18%)</td>
<td>0</td>
</tr>
<tr>
<td>Male</td>
<td>4 (36%)</td>
<td>4 (30%)</td>
<td>2 (18%)</td>
<td>0</td>
</tr>
<tr>
<td>Female</td>
<td>2 (18%)</td>
<td>5 (40%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>9 (82%)</td>
<td>2 (18%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 Severe aplastic anemia (SAA)</td>
<td>5 (45%)</td>
<td>3 (23%)</td>
<td>1 (9%)</td>
<td>0</td>
</tr>
<tr>
<td>2 Immunodeficiency</td>
<td>1 (9%)</td>
<td>3 (23%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3 Severe infections</td>
<td>2 (18%)</td>
<td>4 (30%)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Summary/Conclusions: TA-TMA is a severe endothelial dysfunction syndrome for which, beyond the complement inhibitor ecuclizumab, treatment is not well established. Defibrotide has proven to be safe and effective in sinusoidal obstruction syndrome. Here, we provide encouraging evidence suggesting that defibrotide, as monotherapy or in combination with other agents, may also have a role in the treatment of TA-TMA. Our data show complete resolution of TA-TMA in two thirds of cases, and even higher in those with early treatment and early onset forms of the disease. Validation of single-center experience in prospective controlled studies should be warranted.

E1542
PRE-TRANSPLANT COMORBIDITY AS AN OUTCOME PREDICTOR IN HEMATOPOIETIC CELL TRANSPLANTATION FOR SEVERE APLASTIC ANEMIA
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Background: In the context of allogeneic hematopoietic cell transplantation (allo-HSCT), comorbidities are an important risk factor. Use of the hematopoietic cell transplantation-specific comorbid index (HSCT-CI), which was modeled to effectively capture comorbidity and predict post-transplant outcomes, HSCT-CI had been evaluated in a cohort of patients with a variety of hematologic malignancies. However, it was not validated in a cohort of adult patients with non-hematologic malignancies.

Aims: We performed multi-center retrospective study to validate the prognostic impact of HSCT-CI on transplant outcomes in a cohort of aplastic anemia patients undergoing allo-HSCT.

Methods: We applied the HCT-CI to 140 patients with severe aplastic anemia (SAA) who underwent allogeneic HCT at the Asan Medical Center, Seoul, and Haeundae Paik Hospital, Busan, Korea between April 1995 and March 2013. Required data were retrieved from Asan medical center and Haeundae Paik Hospital BMT Registry Database. We stratified the patients based on comorbidities, as assessed by HCT-CI. Post-transplant outcomes were evaluated in terms of overall survival (OS) and event-free survival (EFS). Event was defined as graft failure including primary and secondary, relapse, donor lymphocyte infusion, and death.

Results: The median age of including patients was 31 year-old (range, 31-61 year-old) and male was 81 patients (58%). HCT-CI score was 0 in 92 patients (65.0%), 1-2 in 34 (24.3%), and ≥3 in 14 (10.2%). The most prevalent comorbidity captured by the HCT-CI was infection (n=20, 14%) followed by moderate/severe hepatic comorbidity (n=10, 7%). During a median surviving post-HCT follow-up period of 45.5 months (range, 4.1-178.4 months), 32 patients (24%) died and 20 (14%) experienced primary or secondary graft failure. The 10-year probability of OS and EFS was 73.4% and 68.3%, respectively. OS and EFS was significantly different according to HCT-CI score; the OS for HCT-CI 0, 1-2, and ≥3 at 4 years was 86.1%, 84.1% and 68.6%, and 60.6% and 65.6%, respectively (P<0.001, respectively) and early-onset TA-TAM (9/11, 82%) vs 2/6, 33%; P=0.046). Multivariate analysis after adjustment for other variables demonstrated that higher HCT-CI score were associated with increased OS and EFS as judged by increasing hazard ratio compared to patients with HCT-CI score of 0 (Table 1).

Table 1.

<table>
<thead>
<tr>
<th>Potential Risk Factors</th>
<th>CR</th>
<th>OS</th>
<th>EFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection</td>
<td>8</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Moderate/severe hepatic comorbidity</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Renal failure</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Central nervous system failure</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Pulmonary failure</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Gastrointestinal failure</td>
<td>1</td>
<td>1</td>
<td>1</td>
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</tbody>
</table>

Summary/Conclusions: In conclusion, our data indicate that the presence of pre-transplant comorbidity assessed by HSCT-CI may predict worse outcomes after allo-HSCT in severe aplastic anemia.

E1543
EFFECTICACY AND SAFETY OF FILGRASTIM BIOSIMILAR COMPARED TO FILGRASTIM ORIGINATOR IN THE STEM CELL MOBILIZATION AND HEMATOPOIETIC ENGRAFTMENT IN PATIENTS UNDERGOING STEM CELL TRANSPLANTATION
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Background: Neupogen® is the original Filgrastim used for peripheral blood stem cell mobilization (PBSM) in patients and donors selected for stem cell transplantation (SCT). Nivestim® is a Filgrastim biosimilar approved for the same indications as Neupogen®.

Aims: To evaluate the efficacy and safety of Nivestim® in the PBSM mobilization for harvesting and hematopoietic SCT.

Methods: Retrospective, controlled, observational study conducted at the University Hospital of Salamanca Hospital between JAN08 and DEC15. The study included 365 patients candidates for ASCT and 217 healthy siblings donors for Allo-SCT who underwent PBSM mobilization. Neupogen® (Amgen Europe BV, Breda, NL) was administered for mobilization at standard doses until SEP2012, while Nivestim® (Hospira, Maidenhead, UK) was used from that date. Among PBSM, 145 were mobilized with Nivestim® and 220 the originator Neupogen®. Patient characteristics between groups were similar, although lenalidomide was more frequently used in the Nivestim® group, as it corresponds to more recent transplants. The mean number of CD34+ cells/µl in the peripheral blood after 4 days of mobilization treatment was not significantly different (Neupogen® 73±32 vs Nivestim® 73±31, P=0.89), but the mean of the total CD34+ collected cells was 4.75, SD=4.41 in the Neupogen® and 6.35±6.42 in Nivestim® group (P=0.01), with a larger number of apheresis procedures needed in the Neupogen® group (1.39, SD=0.65 vs 1.24, SD=0.45, P=0.02). The mobilization failure rate was slightly higher with Nivestim® (22%) than with Neupogen® (13%; P=0.04), although it was attributed to a more frequent use of lenalidomide. Most patients underwent ASCT: 87% and 92% in patients in the Neupogen® and biosimilar groups, respectively. There were no statistically significant differences in hematopoietic recovery and trans-
plant-related toxicity. The median hospitalization time (20, range 14-70 vs 20, range 14-53, p=0.72) and the consecutive number of re-admissions after discharge (27% vs 35%, p=0.35) were also similar between Neupogen® and Nivestim® groups. In the group of HEALTHY DONORS, 95 were mobilized with Neupogen® and 122 with Nivestim®. Donor characteristics were equivalent between groups, and no severe adverse events were registered in any of them. Mean of CD34+ cells collected/kg of recipient body weight was 7.62×10^6/ kg SD=3.45×10^6 for Nivestim® vs 6.26×10^6 SD=2.71×10^6 Neupogen® (p=0.002), but the minimal target cell dose (2×10^6/kg) was collected in all donors. 8.5% of donors mobilized with Nivestim® failed to achieve the optimal cell dose (4×10^6/kg) compared with 13% in the Neupogen® group (p=0.25). All recipients were successfully transplanted. All donors for haploidentical transplants (N=25) were mobilized with Nivestim®; none with Neupogen®. There were no other transplant differences. Platelet and neutrophil engraftment were comparable between the two groups, as well as transfusion requirements and infectious complications after transplant. The incidence of grade 1 to 4 acute graft-versus-host disease was not different (Nivestim®65.5% vs Neupogen® 67.7%; p=0.7). The hospitalization period was similar in Neupogen® and Nivestim® groups, (30 days, range 16-102; 30 days, 16-136, respectively).

Table 1.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Neupogen®</th>
<th>Nivestim®</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45+ cells/µL in peripheral blood, median (range)</td>
<td>3.05 (1.91-5.19)</td>
<td>3.10 (1.85-4.79)</td>
<td>0.29</td>
</tr>
<tr>
<td>Median donor age, n=17 (range)</td>
<td>51 years (range 25-71, n=17 over 60)</td>
<td>51 years (range 25-71, n=17 over 60)</td>
<td>0.7</td>
</tr>
<tr>
<td>Citrate related toxicity</td>
<td>21%</td>
<td>26%</td>
<td>0.5</td>
</tr>
<tr>
<td>Common complication of the apheresis procedure (52%)</td>
<td>34%</td>
<td>50%</td>
<td>0.2</td>
</tr>
<tr>
<td>Mean (SD) of CD34+ cells collected/kg of recipient body weight</td>
<td>7.62×10^6 (3.45×10^6)</td>
<td>6.26×10^6 (2.71×10^6)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Summary/Conclusions: Although prospective data are still required, our study supports that the use of the Filgrastim biosimilar Nivestim® has a similar efficacy and safety as mobilization agent compared with the originator Neupogen®.

E1544

PERIPHERAL BLOOD STEM CELL DONATION IN OLDER SIBLING DONORS: IS IT SAFE?

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Background: The introduction of reduced intensity conditioning regimens has led to an increase in allogeneic haematopoietic stem cell transplantation (HSCT) in older patients with a consequent increase in age of family members who are asked to donate HSCs for them. Such donors are expected to have more comorbidities than younger donors and careful assessment of their suitability to donate is required.

Aims: To analyze long-term results DLI in early posttransplant MC and in relapsed patients after allo-HSCT.

Methods: The study included 61 patients of whom DLI with interleukin 2 (IL-2) was administered at the National research center for Hematology from 2011 till 2016. DLI with IL-2 was administered for patients with MC, more than 10-15% recipient DNA (n=26). A median age was 33 years old (19-54 years). Eight were males, 20 – females. There were AML (n=17), ALL (n=4), MDS (n=2), CML/MPN (n=3). Before allo-HSCT complete remission had in 20 patients and 6 had relapse/progression disease. Patients received allo-HSCT from related (n=20) or unrelated (n=6) donor. The intensity of conditioning was mainly reduced intensity (n=15) rather than myeloablative conditioning (n=11). Bone marrow (BM) as a graft source was used in 20, PBSC – 6. DLI was started at low dose 1×10^6CD3+ per kg. Every following dose of infusion CD3+ increased until 5×10^6CD3+ per kg. Number of infusions depended on achievement 100% donor chimerism. Thirty five patients with relapse after allo-HSCT (AML, n=27, ALL, n=5, MDS, n=2, CML, n=1) were administered DLI with IL-2. Number DLI was 1 or 5 in different cases. Complete remission before allo-HSCT had in 25 patients and 10 had relapse/progression disease. 33 patients received chemotherapy and after chemotherapy on 7 days DLI was using an escalating dose following infusions. Two patients received DLI with IL-2 without chemotherapy. A median age was 33 years old (19-60 years). 14 were males, 21 – females. Stem cell source was BM and (PBSC) in 22 and 13 of the cases, respectively. Patients were transplanted from related (n=17) and from an unrelated donor (n=18). Condition regimen was MAC (n=7), RIC (n=28). Bone marrow as a graft source was used in 22, PBSC – 13.

Results: A median follow up was 5 months (0.3-63). A median time between allo-HCST and DLI was 3 months (1.5-64). 100% donor chimerism was achieved in 17 patients with MC from 26 (65%). A median number of infusions
was 2 (1-5). There were 5 (19%) graft failures. Acute GVHD appeared in 8 (32%), all of them grade 3; chronic GVHD occurred in 7 (27%). Patients with a MC had better overall survival 77.6% than patients with relapse after allo-HSCT (22%). Remission was achieved in 16 (48%) patients with relapses. However, 5 patients relapsed again. Acute GVHD was developed in 8 cases (22%). Nineteen patients died from relapse and 1 patient died from aGVHD in remission. Two patients died in patients with MC and in patients with relapses was 78.6% and 26.2%, respectively.

**Summary/Conclusions:** The prognosis of hematological malignancies is poor if relapse is established after allo-HSCT. DLI protocol as preventive therapy must be created for improving long-term results in high-risk patients. Prevention is better than cure.

**E1546**

**MEMORY T CELLS DONOR LYMPHOCYTE INFUSIONS AFTER HAPLOIDENTICAL STEM CELL TRANSPLANTATION AS A SAFE PROCEDURE TO IMPROVE T-CELL RECONSTITUTION**

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**Background:** Hematopoietic stem cell transplantation (HSCT) is a potential curative treatment for patients with hematologic malignant diseases. Haploidentical transplantation with extensive ex vivo T cell depletion of the graft, has demonstrated to prevent graft versus host disease (GVHD), but the major disadvantage has been the development of graft failure, relapse and infections due to delayed immune reconstitution. A selective T cell depletion method that removes T naive cells CD45RA+ in haploidentical donors, which are responsible for GVHD, as well as preservation of memory T cells CD45RO, is a novel therapy that may provide functional T cells with anti-infection, anti-leukaemia and anti-rejection properties.

**Aims:** We describe the outcome of CD45RA+ cell depletion of donor lymphocytes infusions, in patients with hematologic diseases with mixed chimerism, severe infections and high risk of relapse after hematopoietic stem cell transplantation.

**Methods:** Patients with hematologic diseases with poor prognosis who lacked an HLA matched donor were included. The recipients received a CD45RA-depleted haploidentical transplantation, on day 0 they received a first graft with a median CD34+ cell dose of 6.44x10^6/Kg (range 5x10^6/Kg-9x10^6/Kg), on day +1 they received a CD45RA-depleted graft. After transplantation studies of chimerism, quantification of lymphocyte subsets as well as control for viral infections were made to all patients.

**Results:** We present the results of six patients with a median age of 11 years (range 7-15 years), diagnosis included B-Cell acute lymphoblastic leukemia (n=2), T cell acute lymphoblastic leukemia (n=1), acute myeloblastic leukemia (n=2), aplastic anemia (n=1), these patients received a selective CD45RA-depleted haploidentical transplantation. During the follow up after HSCT, three patients had persistent lymphopenia, four patients developed infections caused by CMV, norovirus, HHV-6, BK virus and toxoplasma, one patient had increasing infections and had one graft failure. These patients were treated with infections of 16 aliquots of cryopreserved CD45RO+ haploidentical donor lymphocytes, the CD45RA+ cells depletion was made using the clinMACS system. The median dose of CD45RO+ cells was 1.02x10^7/Kg, starting at a dose of 2x10^7/Kg every 10 days (range 1.02x10^7-2.05x10^7/Kg) and CD45RA+ cell dose was a median of 0.004x10^7/Kg (range: 0.1-6.1x10^7/Kg). All the procedures were well tolerated, neither adverse events nor GVHD were noticed. After the DLI, a progressive increase in T cells count were observed.

**Summary/Conclusions:** In our experience DLI enriched for CD45RO+ memory T Cell is a promising and safe strategy for patients with severe viral infections and risk of relapse after haploidentical HSCT, these cells has demonstrated to trigger the CD4 and CD8 T cell reconstitution, which will help reduce risk infection with a low risk of GVHD. However further studies are needed in order to support this therapy.

**E1547**

**FLAG REGIMEN WITH IDARUBICINE AS CYTOTHERAPY REDUCTION BEFORE ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH REFRACTORY ACUTE MYELOID LEUKEMIA**

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**Background:** Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only curative option for patients with refractory acute myeloid leukemia (AML). However, allo-HSCT with standard conditioning regimen could merely achieve a long-term survival of 20% and the key problem is the high relapse rate even after transplantation.

**Aims:** We have evaluated the safety and efficacy of new conditioning regimen with sequential intensive chemotherapy (FLAG-IDA) followed by conditioning of Flu-Bu(3).

**Methods:** The study was designed and developed in two separate transplantation centers in Rui Jin Hospital (RJH, Shanghai) and Institut Paoli-Calmettes (IPC, Marseille) respectively. A total of 47 refractory AML patients with median bone marrow blast of 30% (1-90%) and age ≤65 years (16-62) were enrolled. Thirteen patients received transplantation with mobilized peripheral blood stem cells (PBSC) from HLA-matched sibling donor while 18 and 16 with matched unrelated or haplo-identical donors. All patients received FLAG + 3-days idarubicine (12mg/m² in RJH or 10mg/m² in IPC) and then received Flu-darabine (5 days) with IV Busulfan (3-days) with a 7-day interval. The GVHD prophylaxis regimens were CsA+MMF+ATG (RJH) or post-cyclophosphamide (IPC).

**Results:** With a median follow-up of 8 months (1-70m), a total of 14 patients relapsed with a median time of relapse at 4.8 months (2.1-18.1) and most of the patients relapsed within 12 months after HSCT (2.8 and median 19 days). A total of 24 patients died due to relapse (n=12) or non-relapsed mortality (NRM, n=12). The estimated 3-year relapse rate (RR) and NRM were 42.0±9.2% and 25.9±6.5% respectively. The estimated 3-year OS and DFS were 43.6±7.8% and 42.2±8.7%. In the primary multivariate analysis (including age, cycles of pre-transplantation chemotherapy, bone marrow blasts, cytogenetics and treat- ment center), only bone marrow blast ≥35% and age over 40 were associated with disease-free survival and relapse respectively while there was no significant difference between RJH and IPC in terms of transplantation outcome in univariate analysis.

**Summary/Conclusions:** Our primary data demonstrated a promising outcome with FLAG-IDA chemotherapy as debulking therapy sequential with Flu-Bu3 conditioning regimen in patients with refractory AML and clinical trial with larger patients cohort is warranted.

**E1548**

**STUTTER PCR PRODUCTS MAY NOT INTERFERE WITH STR BASED CHIMERISM MONITORING AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION**

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**Background:** Chimerism analysis is one of the main methods to monitor the bone marrow engraftment or disease relapse after allogeneic bone marrow transplantation. Routine test is based on differences in the length of short tandem repeats (STR) from the donor and the recipient. However, chimerism estimation is complicated by stutter PCR peaks appearing due to irregular DNA polymerase activity. Generally, these sequences are 4 nucleotides shorter than a specific marker and may concur with a specific sequence of recipient’s DNA hindering chimerism estimation based on that locus. This problem seems to be especially serious in case of a sex-matched sibling BMT when most of the alleles for donor and recipient are the same. One may suggest to limit the use of these markers for the cases with stutter-bands comparable with donor allele peak height. Therefore, the absence of “stutter-peaks free” markers hides mixed chimerism estimation at the point of low recipient hematopoesis output.

**Aims:** To identify the contribution of stutter-bands to the total amount of PCR-product and to derive universal formulas for the chimerism calculation excluding stutter percentage.

**Methods:** Genomic DNAs of donors and patients were isolated from bone marrow samples. Chimerism was assessed by the STR-PCR analysis (polymerase chain reaction with a panel of primers for loci of short tandem repeats) using AmpFISTR Plus multiplex kit for amplification of 19 polymorphic STR-markers and amelogenin loci. The fragment analysis was performed on a 3130 Genetic Analyzer. The data processing was accomplished using GeneMapper v4.0 software. Informative loci were chosen beforehand comparing pretransplantation

Figure 1.
patient DNA and donor DNA. The percentage of donor chimerism as well as stuffer percentage was calculated using standard formula.

**Results:** Fifty transplant cases with stuffer peaks were evaluated: 18 heterozygous; 15 heterozygous with both alleles showing detectable stuffer; 17 heterozygous with one stuffer visible only. Stuffer percentage and standard deviation were calculated in each case for donor DNA sample and for four bone marrow DNA samples from recipient with established complete donor chimerism taken during the time. It was found that the contribution of the stuffer-peaks into the total amount of product ranges from 1.2% to 11% (SD was no more than 1.5% for each locus) for markers with appreciable stuffer-bands and seems to be locus-specific constant for each patient. Assuming the stuffer percentage as a locus- and 13.63% in the constant (for the same PCR conditions) we derived a formula for recipient DNA percentage: Actual recipient’s%=(apparent rec./total DNA ratio - stuffer/total DNA ratio)∗100% (special formulas for hetero- and homogenous on fig. 1). To test these formulae the panel of DNA samples with mixed chimerism from 50 to 97% estimated by independent “stutter-free” markers appeared to be the same (SD<1%).

**Summary/Conclusions:** The use of formulae described may circumvent the absence of the “stuffer-free” informative markers for mixed chimerism estimation.

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**E1550**

**PERIPHERAL BLOOD STEM CELL (PBSC) HAPLOIDENTICAL TRANSPLANTATION VERSUS MISMATCHED UNRELATED DONOR TRANSPLANTATION: A SINGLE UK CD34+ STUDY CENTRE EXPERIENCE.**


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**Background:** Haploidentical (Haplo) and matched unrelated donor transplantation (MMUD) are increasingly used as an alternative when an HLA-matched unrelated donor is unavailable. Recent collaborative and single centre studies suggest that haploidentical donor outcomes are comparable to unrelated donor outcomes in the T cell-replete setting.

**Aims:** In this single centre review, we aimed to compare outcomes of T cell-replete haploidentical allogeneic stem cell transplantation with mismatched unrelated donor allogeneic stem cell transplantation.

**Methods:** From January 2010 to December 2015, 38 patients underwent T cell-replete HLA-matched haploidentical transplantation with post transplantation cyclophosphamide given on days +3 and +4 given as graft versus host disease (GVHD) prophylaxis. These were retrospectively compared with 45 patients underwent single HLA-locus mismatched unrelated donor transplantation with alemtuzumab as GVHD prophylaxis. Data was censored at time of last contact in 2016. Analysis was performed using SPSS v23.0.

**Results:** The median recipient age was similar in both groups; 51 (19-69) years in Haplo and 59 (28-74) years in MMUD transplants, p=0.012; 68.7% of all patients were male. Non-Caucasian ethnicity comprised 63.2% of Haplo versus (vs.) 15.6% of MMUD transplants, p<0.001. Myelodysplasia (MDS)/acute myeloid leukaemia (AML) was the commonest transplant indication in both groups (60.5% of Haplo and 93.6% of MMUD transplants). The disease risk index (DSI) in this subgroup was overall low/intermediate in 69.2% and high/high in 26.2% (unknown in 4.6%). Reduced intensity conditioning was used in all but two Haplo (4.6%) and 4 MMUD transplants. Patients were followed up for a median of 544 days with a similar 2-year overall survival of 61.5% (95% confidence interval, CI, 52.4 – 69.3%) and 58.1% (95% CI 48.8-66%) and 3-year overall survival of 56.4% (95% CI 45.8 – 65.6%) and 48.9% (95% CI 41 – 56.2%) in Haplo and MMUD transplants respectively, p=0.67. Overall progression free survival (PFS) at 2 years was 53.3% (95% CI 44-61%) and 40.1% (95% CI 34-46%) in Haplo and MMUD transplants respectively, p=0.31. In those with MDS/AML, the 2-year progression-free survival was 62.4% (95% CI 49-75%) in Haplo vs 38.5% (95% CI 33-43%) in MMUD transplants, p=0.1. In Haplo and MMUD transplants, the 3-year cumulative incidences of non-relapse mortality were 25.5% (95% CI 12-41%) and 31.2% (95% CI 18-45%) respectively, p=0.61. Survivors and non-survivors were comparable for sex, age and organ failure criteria.

**Conclusions:** Our data shows that in high-risk haploidentical transplantation when compared with T cell-deplete mismatched unrelated donor transplantation showed high engraftment rates, low rates of severe acute and chronic GVHD and comparable overall survival, non-relapse mortality and relapse rates. We suggest that T cell-replete haploidentical transplantation is a safe and acceptable alternative when a matched unrelated donor is unavailable.

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**E1549**

**INTRODUCING PLERIXAFOR TO IMPROVE MOBILIZATION IN MULTIPLE MYELOMA PATIENTS WHO BEHAVE AS POOR-MOBILIZERS IS COST-EFFECTIVE CONSIDERING THE WHOLE MOBILIZATION AND TRANSPLANT PROCEDURE.**

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**Background:** Plerixafor, a CXCR4-antagonist, is efficient to improve CD34+ cell mobilization and collection in candidates for autologous transplantation who behave as poor-mobilizers. The cost of the drug is however of concern.

**Aims:** To compare costs and effectiveness of pleixafor-free and pleixafor-replete management strategies for multiple myeloma patients who behaved as poor-mobilizers after adequate administration of a standard rhG-CSF mobilization regimen.

**Methods:** Sixty patients diagnosed with multiple myeloma were consecutively identified during years 2009-2011, immediately before and after EMA granted marketing authorization for pleixafor. Poor-mobilizers were defined as having circulating CD34+ cell counts below 20x10⁶L⁻¹. Plerixafor was introduced or not as a result of the attending physician’s decision, reflecting progressive changes in medical practices over this transitional period. The historical and study groups were matched over four criteria: disease stage at diagnosis, age, gender and number of chemotherapy treatments received before mobilization. Two cost-effectiveness analyses were conducted: the primary CEA looked at the criterion “collecting at least 2x10⁶ CD34+ cells”; a secondary CEA looked at the criterion “successful autologous transplant administered”. Detailed micro-costing evaluations (2015 figures) did not or did include transplantation costs for the first and second CEA respectively.

**Results:** The two groups were similar in terms of age, sex distribution, disease characteristics or previous treatments. 27/30 and 26/30 patients proceeded to the minimal target number of 2x10⁶ collected CD34+ cells/kg was identical (8.162€ respectively). The main cost drivers were: collection of peripheral blood stem cells (PBSC) and subsequent autologous transplantation with 18.7% of the total cost in poor-mobilizers, and 21.1% of the total cost in pleixafor-free mobilizers. The pleixafor-replete strategy showed a considerable reduction in costs: 7.3%, 6.8% and 3.1% relative to poor-mobilizers in the primary CEA, and 12.1%, 14.8% and 6.3% in the secondary CEA respectively. The CEA was incremental, meaning that pleixafor-replete mobilization is cost-effective, based on the assumption that pleixafor is as effective as the standard mobilization procedure.

**Conclusions:** Plerixafor-free mobilization is a cost-effective strategy for multiple myeloma patients who behave as poor-mobilizers. The cost of the drug is however of concern. Recent collaborative and single centre studies suggest that pleixafor-free mobilization in candidates for autologous transplantation with 30-50% of allogeneic transplantation shows ABO incompatibility, but its clinical impact is controversial. It’s accepted in solid organ transplant; thereby, up to 30-50% of allogeneic transplantation with ABO incompatibility1, but its clinical impact is controversial. It’s accepted as a safe and acceptable option.
(RBC) recovery, neutrophil and platelet engraftment, pure red cell aplasia (PRCA), acute GVHD, relapse and event free survival (EFS).

Methods: We retrospectively studied allogeneic transplants performed from January 1, 2013 to December 31, 2016. We collected the baseline variables reflected in Table 1 and analyzed the incidence of HE, neutrophil and platelet engraftments, RBC recovery, PRCA (defined as anemia with transfusional requirement and reticulocytes <1% in day +60 without other cytopenias), acute GVHD, relapse of the background disease and survival (at 6, 12 and 24 months) in the ABO compatible groups (ABOc) and in the incompatible (ABOi), the latest divided into major, minor and bidirectional disparity.

Results: A total of 133 transplants were included, with a mean follow-up time of 16.4 months. The median age was 52 years and there were 79 males and 54 females. Diagnoses were mainly AML (n=72), ALL (n=19) and NHL (n=11) (see Table 1). 60 received low intensity and 73 myeloablative regimens. They were HLA identical (n=44), unrelated donor (n=50), haploidentic (n=38) and cord (n=1) and, in most cases, hematopoietic progenitors were obtained from mobilized peripheral blood (90.2%), 44.3%. (n=59) presented some type of ABOi: major (n=26), minor (n=25) and bidirectional (n=8). The product was processed in order to prevent hemolysis in only 7 cases (red cell depletion in 4 and deplasmatization in 3). There were 23 hemolytic (18 immediate and 5 delayed) -mostly mild- events, which appeared predominantly in patients with ABO-incompatibility (38.98%)- 50% in major disparity, 28% in minor and 37.5% in bidirectional- vs ABOc (2.7%) and this difference was statistically significant (p<0.0001). No differences were observed in the neutrophil graft between the ABOc group and the ABOi group, nor in the platelet engraftment; in contrast, we found a statistically significant effect on the time to erythrocyte recovery (mean: 49.94 days in ABOc vs 24.69 in ABOi; p=0.032). Only 6 cases of PRCA were documented (all in ABOi). The occurrence of acute GVHD did not differ significantly among the groups (52% in ABOc vs 53.5% in incompatibles) nor in its severity. We have not found differences either in the rate of relapse (24.6% vs 19.1%) nor in the survivals at 6, 12 or 24 months (66.1% vs 78.8%, 48.2 vs 47.2% and 38.4 vs 39.4%, respectively).

Summary/Conclusions: In our study ABO-mismatched transplants have shown a greater number of hemolytic events and red cell aplasia, as well as a greater delay in achieving erythrocyte recovery. However, we have not found an association with delayed neutrophil and platelet recoveries, increased acute GVHD, relapse or worse OS in the ABO incompatible group, in keeping with most previous reports*, although the absence of effect might be as well be related to an insufficient study power due to low sample size.

References

Table 1.

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Figure 1.

Low blood concentration of Tacrolimus can be a risk of graft failure after cord blood transplantation.

Aims: Several biological mechanisms may contribute to graft failure. Immunological rejection of the graft is known as a major cause of graft failure. Graft failure may also be caused by sepsis, viral infections, drug toxicity and so on. These events have been frequently occurred just before engraftment, and we often experience fluctuation of blood levels of immunosuppressive drugs. Here, we analyzed an association between blood levels of Tacrolimus (Tac) before neutrophil engraftment and neutrophil engraftment.

Methods: Between January 2011 and June 2016, 76 patients received single-unit CBT at our institutions. We analyzed 59 patients for whom Tac was used for GVHD prophylaxis including Tac and Mycophenolate mofetil (MMF) combination (n=26) and Tac with an additional short Methotrexate (sMTX) (n=33). Sixteen patients who underwent second or third CBT and a patient for whom Tac was not used for GVHD prophylaxis were excluded. We also excluded a patient whose Tac concentration we didn’t check more than two times a week. Tac was started at a dose of 0.02mg/kg/day by continuous i.v. infusion. Tac blood concentrations were monitored at least three times a week before engraftment, and dosages were adjusted to maintain serum levels about 10-20 ng/ml.

Results: Of the 59 patients, 48 patients achieved neutrophil recovery at a median of 22 (range 13-35) days. Two patients died before engraftment from severe PIR and active infection. Nine patients (18.6%) experienced graft failure. Patients who could maintain Tac level above 12ng/ml during the second week after CBT (Tac high group) had an incidence of graft failure of 4.8%, which was significantly lower than the 26.3% seen in the other patients (Tac low group) (p<0.01). Patients for whom Tac and MMF were used (MMF group) had an incidence of graft failure of 3.8%, which was significantly lower than the 36.4% seen in the other patients for whom Tac with an additional sMTX (MTX group) for GVHD prophylaxis (p<0.01). Combined of these factors, the patients of Tac low group and MTX group had an incidence of graft failure 40.9%, which was significantly higher than the 5.4% seen in the other patient including Tac high group and MMF group even if the patient were included of Tac low group.

E1533

THE EXPRESSION OF TOLL-LIKE RECEPTORS GENES IN PATIENTS WITH LYMPHOID MALIGNANCIES AFTER AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

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Background: Peripheral blood stem cell transplantation (PBSCT) is one of the main strategies for the treatment of malignant hematological diseases. Toll like receptors (TLRs) are present on various immune cells including natural killer cells, monocytes, macrophages, T lymphocytes and B lymphocytes. Ten different TLRs have been evaluated in humans. TLRs play a central role in immune surveillance and in the initiation of the inflammatory response. The expression of TLRs genes and their association with outcome in patients treated with PBSCT remains undecided.

Aims: The objective of the current study was to investigate association between expression of TLRs genes and hematopoietic recovery and rate of infections in patients treated with PBSCT.
Methods: The evaluation of TLRs expression genes were performed in 40 patients who underwent PBSCT. The median age of patients was 54 years (range: 25-65 years). There were 15 patients with multiple myeloma (MM), 20 patients with non-Hodgkin lymphomas (nHLs) and 5 patients with Hodgkin lymphoma (HL). Peripheral blood samples were taken before megachesemotherapy with autologous stem cell transplantation and at time of hematopoietic recovery in patients after PBSCT. Relative expression of TLR receptors was assessed by real-time PCR using inventoried TaqMan® Assays from Life Technologies/ThermoFisher. Beta glucuronidase (GUSB) served as endogenous control. Reaction was performed in 7500 Real Time PCR instrument (LifeTechnologies) using Gene Expression MasterMix (LifeTechnologies/ThermoFisher). Comparative Ct method (**) was used to compare expression among patients and with healthy controls. Statistical analysis was conducted using STATISTICA 12 software (StatSoft, Polska). For quantitative variables arithmetic means (X) and standard deviations (SD) of estimated parameters were calculated in the analysed groups. Distribution of variables was examined using graphs of Leiflerfe and W-Shapiro-Wilk. In cases of independent quantitative variables with the normal distribution the statistical analysis took advantage of t test for unlinked variables. In cases of variables manifesting distribution distinct than the normal one, for independent quantitative variables U test of Mann-Whitney was used. For dependent quantitative variables of the normal distribution, the t test for linked variables was applied. In cases of quantitative dependent variables with the distribution distinct from normal, the pair sequence test of Wilcoxon was applied. In order to define a relationships between the studied variables, correlation analysis was performed. Results: The mRNA expression of TLR2 and TLR9 was significantly higher in patients after PBSCT than before PBSCT procedure (ΔCt TLR2 1,4209±1,0461 vs 1,7877±1,4974 and ΔCt TLR9 117,853±1,0461 vs 289,788±271,98) (p<0,05). We observed that expression of TLR9 was significant higher in patients with bacterial and fungal infection after PBSCT in comparison to group without infection after PBSCT (ΔCt TLR9 117,853±1,0461 vs 289,788±271,98) (p<0,05). Moreover we found significant positive correlation between expression of mRNA of TLR9 and neutrophil recovery after PBSCT (r=0,4075; p=0,023).

Summary/Conclusions: In conclusion our findings suggest that TLRs could be useful markers in outcome in patients treated with PBSCT. This observation should be validated by larger study.

E1554
TIMING OF DEFIBROTIDE INITIATION POST- DIAGNOSIS OF HEPATIC VENO-OCCCLUSIVE DISEASE/SINUSOIDAL OBSTRUCTION SYNDROME AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION: EXPANDED ACCESS PROGRAM FINAL DATA
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Background: Hepatic veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS) is an unpredictable, potentially life-threatening complication of hematopoietic stem cell transplantation (HSCT). VOD/SOS with multi-organ dysfunction (MOD) may be associated with >80% mortality. Defibrotide is approved in Europe to treat severe hepatic VOD/SOS post-HSCT in the European Union and to treat hepatic VOD/SOS with renal and/or pulmonary dysfunction post-HSCT in the United States. Prior to approval in the United States, defibrotide had been available via an expanded-access program.

Aims: To perform an exploratory post hoc analysis of final data from the expanded access program on the impact of delayed timing of initiation of defibrotide after diagnosis of VOD/SOS in HSCT patients.

Methods: In an expanded-access study, patients diagnosed with VOD/SOS (per Baltimore criteria, modified Seattle criteria or biopsy) with or without renal/pulmonary MOD after HSCT or chemotherapy received defibrotide 25mg/kg/day in four divided doses for a recommended ≥21 days after patients provided informed consent. For these exploratory analyses, Day +100 survival rates in HSCT patients were examined post hoc by time from VOD/SOS diagnosis to start of defibrotide for (1) all patients before/after days 1, 2, 3, 4, 7, and 14, using Fisher’s exact test and (2) patients starting defibrotide on a particular day (including patients ≥15, by Cochran-Armitage test for trend across days. Causes of treatment delay were not assessed.

Results: In the final dataset, timing of initiation date was available for 1000 HSCT patients (512 with MOD) who received ≥1 dose of defibrotide. In 31.0% of all HSCT patients, defibrotide was started the day of diagnosis; in 92.9%, by Day 7. In the population-wide analysis of initiation before/after days 1, 2, 3, 4, 7, and 14 post-diagnosis in both the overall group and MOD subgroup (Figure 1), earlier initiation was associated with significantly higher Day +100 survival rates for all days (P<0.001), except Day 14 (2.6% of patients started defibrotide after Day 14). The trend test for particular initiation days also showed a significant trend over time for higher Day +100 survival with earlier defibrotide initiation post-diagnosis in both the overall HSCT group and MOD subgroup (P<0.001). Adverse events (AEs) and serious AEs occurred in 70.8% and 53.4% of patients, respectively. Other than VOD/SOS and MOD, the most common AE was hypotension (11.7%) and most common serious AE was respiratory failure (7.3%).

Figure 1.

Summary/Conclusions: In this exploratory analysis of final study data, earlier defibrotide initiation post-VOD/SOS diagnosis significantly improved Day +100 survival, confirmed by the Cochran-Armitage test (P<0.001). No specific day provides a clinically meaningful cutoff for better Day +100 survival, suggesting that later intervention retains value if treatment must be delayed.

Support: Jazz Pharmaceuticals.

E1555
RED BLOOD CELL DISTRIBUTION WIDTH (RDW) AS AN ACUTE GRAFT VERSUS HOST DISEASE PREDICTOR MARKER IN ALLOGENIC STEM CELL TRANSPLANTATION
B. Robredo1, F. Sartori1, M.A. Duran1, A. Gutierrez1, A. Sampol1, L. Lo Riso1, J.M. Sanchez-Raga1, B. Lopez Andrade1,*, 1Hematology, Hospital Universitario Son Espases, Palma Mallorca, Spain

Background: The red blood cell distribution width (RDW) is a common parameter for measuring anisocytosis in the study of anemia. Recently it has been regarded as a surrogate marker for adverse outcome in several diseases. Acute graft-versus-host disease (GVHD) is a common complication of allogeneic hematopoietic cell transplant (allo-HSCT) which is related to inflammation in the context of damage of the host tissue and the release of inflammatory cytokines. We decided to study the utility of this potential inflammatory marker in the setting of GVHD in the allo-HSCT.

Table 1.

| N | GENDER | MEAN AGE | MEAN YEARS OLD | MEAN MIRG | MEAN MEDIAN | MEAN MEDIAN | MEAN MEDIAN | MEAN MEAN | MEAN MEAN | MEAN MEAN | MEAN MEAN | MEAN MEAN | MEAN MEAN | MEAN MEAN | MEAN MEAN | MEAN MEAN | MEAN MEAN | MEAN MEAN | MEAN MEAN |
| 102 | Male | 30 | 31 | 42 | 42 | 42 | 42 | 42 | 42 | 42 | 42 | 42 | 42 | 42 | 42 | 42 | 42 | 42 | 42 |

GVHD

- VHS 62 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |

Table 1.

| N | GENDER | MEAN AGE | MEAN YEARS OLD | MEAN MIRG | MEAN MEDIAN | MEAN MEDIAN | MEAN MEDIAN | MEAN MEAN | MEAN MEAN | MEAN MEAN | MEAN MEAN | MEAN MEAN | MEAN MEAN | MEAN MEAN | MEAN MEAN | MEAN MEAN | MEAN MEAN | MEAN MEAN | MEAN MEAN | MEAN MEAN |
| 102 | Male | 30 | 31 | 42 | 42 | 42 | 42 | 42 | 42 | 42 | 42 | 42 | 42 | 42 | 42 | 42 | 42 | 42 | 42 | 42 |
Aims: RDW values were evaluated at the day of infusion (RDW 0), we chose this point in time to evaluate the tissue injury and inflammation secondary to the conditioning regimen, in order to evaluate if there is a major incidence of GVHD.

Methods: We retrospectively evaluated 103 patients who had undergone allo-HSCT for different indications at our center, with a median follow up of 12.8 months (0-235) at our center. The population consisted of 59 males and 44 females, the median age was 43.7 years. The RDW was collected from the hemogram at the day of the HSCT cell infusion, before it was performed (table 1). The IBM SPSS STATISTICS program was used for all statistical analyses. Differences were considered statistically significant when p<0.05. The median of RDW values in our study was of 16.4 (11.2-38.5). The areas under the receiver operating characteristic (ROC) curves of RDW were ≤18.4 and >18.4 for the selection of the increased RDW cutoff. We evaluated the association of increased RDW (>18.4) with the development of GVHD. A survival analysis of the association of different levels of increased RDW was performed. A subgroup analysis of the Haploidentical HSCT patients (N=13) was also evaluated.

Figure 1.

Results: The presence of increased RDW >18.4 was strongly associated with an increased risk of developing acute GVHD (p=0.009) being present in 80% of the patients. In the haploidentical HSCT subgroup an increased RDW >16 was associated with acute GVHD. (p=0.044). There was no association of chronic GVHD with elevated RDW at day 0 (p=0.563). The survival analysis didn’t found an association of high RDW levels with mortality or survival (p=0.301) but a tendency to an increased survival was show between the RDW level subgroups. (figure 2). Where a higher RDW seems to have a better survival, but this should be evaluated in a wider sample.

Summary/Conclusions: RDW at day 0 is a feasible predictor factor of Acute GVHD, most likely as a secondary surrogate marker of inflammation secondary to the conditioning regimen. The presence of other factors contributing to the RDW increase (secondary to other comorbidities) cannot be ruled out, but by itself RDW it’s an easy and affordable prognosis marker for aGVHD that should be further evaluated.

E1556

COMPARISON OF THE BEEAM CONDITIONING REGIME AND THE BEAM CONDITIONING REGIMEN IN THE AUTOLOGOUS TRANSPLANATION FOR HL AND NHL

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Background: The BEAM has established itself as a standard of care conditioning regimen in the autologous lymphoma HSCT setting for most transplant centres in Europe. Yet however various other regimens are being compared with it in order to achieved better safety profile, better OS and DFS, in order to improve results with chemoresistant and unfavourable patients. One such regimen is the BeEAM (bendamustine, etoposide, cytarabine, melphalan).

Aims: We aimed to compare the efficacy of the BEAM and BeEAM conditioning regimens and to compare there myelotoxicity profile.

Methods: We evaluated retrospectively 114 patients, receiving auto-HSCT at our center. Between 06/09 and 09/16, 31 pts underwent DUCBT - 11 males, 20 females with median age 50 years (range 19-59). Diagnosis was acute myeloid leukemia (AML; n=12), acute lymphoid/mixed phenotype leukemia (n=7), chronic lymphoproliferative disease (n=5), MDS (n=4) or other (n=3). All 31 pts recovered ANC>0.5x10^9/L at median of 20 days (range 14-72). Platelet count reached >20x10^9/L in 26/31 pts at median of 38 days (range 24-188). Platelet count reached >20x10^9/L in 26/31 pts at median of 38 days (range 24-188). Acute GVHD developed in 26/31 pts (84%) and chronic GVHD in 17 of the 26 pts (65%) that survived to day +100. Seventeen pts (55%) remain alive, in contin-

Results: The OS at 2 and 3 years respectively was 86.1%, 86.1% for BeEAM and 78%, 71% for BEAM, the DFS at 3 years was 76.4% in BeEAM and 73.2% BEAM, provided that the differences did not have statistical significance. The CR rate was 63.63% in the BeEAM group versus 50% in the BCU1 group. 22.72% of the patients receiving BeEAM in SD or in diseases progression achieved CR versus 10.86% respectively for the BEAM group. The mean time to hematological recovery for neutrophils was 11.27 days (BeEAM) versus 10.24 days (BEAM) and 12.64 days (BeEAM) versus 11.12 days (BEAM) for platelets.

Summary/Conclusions: BeEAM appears to be a non-inferior alternative conditioning regimen to the standard BEAM, it shows a trend towards higher myelotoxicity, but also a trend towards better short-term results in chemoresistant patients.

E1557

DOUBLE UMBILICAL CORD BLOOD TRANSPLANTATION IN ADULTS: CORRELATION OF ALLELE-LEVEL HLA MATCHING WITH OUTCOME AND WHICH CORD BLOOD UNIT WILL BECOME DOMINANT

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1Leukemia/BMT Program of BC, Vancouver General Hospital, Vancouver, Canada

Background: Umbilical cord blood (UCB) has been used for alternative donor transplantation for the past 3 decades. Graft failure is not uncommon due to higher degrees of histoincompatibility between recipient and UCB units and fewer hematopoietic precursors in the product. To improve engraftment rates, especially in larger (i.e. adult) patients (pts), two UCB units can be used. Double UCB transplantation (DUCBT) is being utilized at many centres although it has been noted that, while both units may contribute to engraftment, only one unit becomes “dominant” – i.e. persists to provide long-term hematopoiesis. A variety of predictors of which unit will become dominant have been suggested, primarily the unit that is more closely HLA-matched or the unit with the highest total nucleated cell (TNC) count.

Aims: To determine the likelihood of engraftment, incidence of GVHD, influence of TNC count and HLA mismatch on survival and selection of the dominant cord following DUCBT in adults with high-risk hematologic disorders.

Methods: A retrospective review was performed of adult pts undergoing DUCBT at the referral centre for British Columbia. Recipients signed informed consents for all clinical trials in which they participated. HLA typing at A, B, C and DRB1 loci was done on all pts using high-resolution allele-level testing (HRT). HRT was available at these loci for both UCB units in 25/31 pts; for the remaining units, class I typing was done by serology. UCB units selected had to be 2/6 match at A, B (serologically) and DRB1 by (HRT). Combined TNC count for the units had to be ≥3x10^10/kg recipient weight. Conditioning was Fludarabine 40mg/m² x4 and TBI 150 cGy x9; GVHD prophylaxis was Tacrolimus/Mycophenolate. Pts received G-CSF 300 mcg s.c. daily from day +1. Outcomes were compared using Fisher’s exact test.

Results: Between 06/09 and 09/16, 31 pts underwent DUCBT - 11 males, 20 females with median age 50 years (range 19-59). Diagnosis was acute myeloid leukemia (AML; n=12), acute lymphoid/mixed phenotype leukemia (n=7), chronic lymphoproliferative disease (n=5), MDS (n=4) or other (n=3). All 31 pts recovered ANC>0.5x10^9/L at median of 20 days (range 14-72). Platelet count reached >20x10^9/L in 26/31 pts at median of 38 days (range 24-188). Acute GVHD developed in 26/31 pts (84%) and chronic GVHD in 17 of the 26 pts (65%) that survived to day +100. Seventeen pts (55%) remain alive, in contin-
uous remission at median follow-up of 3 years (range 0.5-7.0). Ten pts (32%) experienced non-relapse mortality from GVHD (5 pts), infection (4 pts) or unknown cause (1 pt). Four pts (13%) have relapsed at 3.5, 10 and 12 months. Outcomes for pts when the best cord unit matched was 0-2 antigen-mismatched (Ag-MM) were superior (8/12 alive and well) to those pts when the best unit was 3 Ag-MM (3/9 alive and well; p=0.20). Unexpectedly, 6/9 pts whose best unit was ≥4 Ag-MM were alive and well. Information on the dominant cord was available on 19 pts (Table 1); in 15/19 pts, the dominant cord was the same or a better HLA match compared to 4/19 with a dominant cord that was an inferior HLA match (p<0.001). However, the TNC was of less importance with the lower TNC unit being dominant as frequently as the higher TNC unit for each HLA match category (Table 1).

### Table 1.

<table>
<thead>
<tr>
<th>HLA Match</th>
<th>HLA Match Higher TNC</th>
<th>Same TNC</th>
<th>Same TNC</th>
<th>Lower TNC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Better (1-2)</td>
<td>34/39</td>
<td>34/39</td>
<td>34/39</td>
<td>34/39</td>
</tr>
<tr>
<td>Same (3-4)</td>
<td>34/39</td>
<td>34/39</td>
<td>34/39</td>
<td>34/39</td>
</tr>
<tr>
<td>Worse (5-6)</td>
<td>34/39</td>
<td>34/39</td>
<td>34/39</td>
<td>34/39</td>
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</tbody>
</table>

Summary/Conclusions: DUCBT is effective in adults with life-threatening hematologic disorders. With current UCB inventories, conditioning therapies and supportive care, graft failure is rare - even in adults. HLA typing between the UCB unit and the patient is a better predictor than the TNC regarding which unit will become dominant. Pts receiving well-matched UCB units (0-2 Ag-MM) may have better outcomes than pts receiving 3 Ag-MM units although successful outcomes can be seen even with a high degree (>4 Ag-MM) of HLA incompatibility.

### CLINICAL ANALYSIS OF HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR 46 ACTIVE RELAPSED AND REFRACTORY ACUTE PEDIATRIC LEUKEMIA

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**Background:** Given the dismal prognosis for relapsed and refractory (R/R) acute leukemia, many physicians discourage offering hematopoietic stem cell transplantation (HSCT) to adults with bone marrow (BM) blasts over 25%. Therapeutic recommendations for pediatric subjects with a similar situation are not available.

**Aims:** With no significant alternative managing options for these patients, more data are required to make an informed and patient tailored decision.

**Methods:** We retrospectively analyzed the preliminary outcome of 46 active R/R acute leukemia patients. From January 16 to 2012 and 2016. Median age at HSCT was 13 years. Active R/R disease was all confirmed by cytogenetics/molecular genetics and aggressive clinical course. Median bone marrow blasts was 46.4% (5-99%). Of note, 27 patients had over 50% blasts in BM. The earliest 13 transplants were conditioned with conventional Bu/Cy or TBI/Cy regimen, thereafter, all received intensified conditioning including FLAG/TBI (N=21), FLAG/Bu/Cy (N=2) and CLAG/Bu/Cy (N=10). Immuno-suppressive agents withdrawal started since day 30 if no acute GVHD occurred. Variety of post-HSCT intervention including donor lymphocytes infusion and intravenous-2 injection were performed to reduce relapse. Median follow-up of the whole cohort is 19 months (3–53 months).

**Results:** Forty-five (97.8%) achieved CR following HSCT. One died of infection before engraftment. All 3 death occurred before 90 day due to relapse. Transplant-related mortality at year 1 was 15.2%. Acute GVHD incidence was 49.3% (grade III 20.4%), chronic 59.5%. Relapse was the major cause of treatment failure and occurred in 28.3% of patients at a median of 1 year post HSCT. Two-year overall survival and leukemia-free survival were 44.8±9.5% and 44.6±8.9%, respectively. Survival of AML patients was superior to those of ALL. Refractory disease or any refractory diseases were equivalent to those with relapsed refractory AML which not seen in ALL. Blast percentage (grade III 20.4%), chronic 59.5%. Relapse was the major cause of treatment failure. There is no standard therapy for relapse after SCT and treatment results are very poor. Treatment options range from supportive care through chemotherapy and donor lymphocyte infusion (DLI) up to a second SCT from the same or a different donor.

**Aims:** To report a retrospective study of 36 patients AML relapsed patients following allogeneic stem cell transplantation in first remission.

**Methods:** Between 2000 and 2016, 130 adults with AML in first CR underwent allo-SCT. We identified 36/130 patients (27%) who had relapsed and proceeded to review the management and outcomes of these patients; the incidence of relapse was 20% and 54% after myeloablative and reduced intensity conditioning, respectively. The median time to disease relapse after allo-SCT was 11 months (range 5-48; 15/36 (41%) of relapsed patients suffered aGVHD grade II-IV or extensive cGVHD. At time of relapse 15/36 (41%) patients were still taking immunosuppressive treatment, which was immediately suspended.

### OUTCOMES OF PATIENTS RELAPSING FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION FOR AML IN FIRST CR: SINGLE CENTER EXPERIENCE

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**Background:** Allogeneic stem-cell transplantation (SCT) is a curative therapy for patients with AML but disease relapse continues to be the most common reason for treatment failure. There is no standard therapy for relapse after SCT and treatment results are very poor. Treatment options range from supportive care through chemotherapy and donor lymphocyte infusion (DLI) up to a second SCT from the same or a different donor.

**Aims:** To report a retrospective study of 36 patients AML relapsed patients following allogeneic stem cell transplantation in first remission.

**Methods:** Between 2000 and 2016, 130 adults with AML in first CR underwent allo-SCT. We identified 36/130 patients (27%) who had relapsed and proceeded to review the management and outcomes of these patients; the incidence of relapse was 20% and 54% after myeloablative and reduced intensity conditioning, respectively. The median time to disease relapse after allo-SCT was 11 months (range 5-48; 15/36 (41%) of relapsed patients suffered aGVHD grade II-IV or extensive cGVHD. At time of relapse 15/36 (41%) patients were still taking immunosuppressive treatment, which was immediately suspended.
Results: The patients was subdivided into three groups according to the salvage treatment received palliative/supportive care (PSC group, n=9, 25%), intensive chemotherapy alone (CHT group, n=18, 50%) and chemotherapy with immunotherapy (donor lymphocyte infusion or second SCT) (IT group, n=9, 25%). Median age at the start of treatment at relapse was 10, 20 and 25 days in the PSC, CHT and IT groups, respectively. In the CHT group, 3 patients (16%) had grade III and 4 (22%) during reinduction chemotherapy. In the IT group, 6 (66%) pts achieved a second CR after chemotherapy and DLI/second allo-SCT and 3 (34%) died of treatment toxicity. In the two patients, sample, median overall survival (OS) was 7 months (range 2-74), being 4, 5, 13 months in the PSC, CHT and IT group, respectively. Estimated 1-year and 2-years overall survival was 10%, 15%, 40% and 0%, 0%, 12% in the PSC, CHT and IT groups, respectively. In our experience, 3 independent factors for a longer OS after chemotherapy and immunotherapy have been identified: the absence of previous acute or chronic GvHD (HR=2.7,p<0.001), a longer interval between the allo-SCT and relapse than 12 months (HR=1.2, p=0.005) and age less than 40 years (HR=1.3, p=0.005).

Summary/Conclusions: This study shows that salvage chemotherapy (with DLI or second allo-SCT) provides the best results and should be offered, whenever possible, to patients with AML who relapse after allo-SCT performed in first CR. Patients undergoing chemotherapy alone had a poorer outcome. Our results emphasize the need to schedule a prospective protocol combining cytoreductive treatments and immunotherapy in patients with AML relapsing after allo-SCT.

E1561

ALLOGENIC STEM CELL TRANSPLANTATION FOR PATIENTS WITH CHEMOREFRACTORY HODGKIN LYMPHOMAS: A RETROSPECTIVE MULTICENTER EXPERIENCE OF THE RETE EMATOLOGICA PUGLIESE (REP)

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1Haematology, Panico Hospital, Tricase, 2Haematology, Policlinico Hospital, Bari, 3Hematology, Mazzoni Hospital, Ascoli Piceno, 4Hematology, “Casa Sollievo” Hospital, San Giovanni Rotondo (FG), 5Haematology, Moscati Hospital, Taranto, Italy

Background: Second-line salvage high-dose chemotherapy and autologous stem cell transplantation (SCT) have become the standard of care for refractory/relapsed Hodgkin lymphomas (HL) leading to durable responses in approximately 50% of relapsed patients and a minority of refractory patients. Patients with refractory HL after autologous SCT generally have poor clinical outcomes with available therapies and by far, allogeneic SCT represents the only strategy with a curative potential.

Aim: The aim of this retrospective analysis was to evaluate the outcome of allogeneic transplantation outcomes patients with HL chemotherapy following last salvage treatment.

Methods: 39 patients with HL who received allogeneic SCT in chemorefractory disease, from 2000 to 2016 were retrospectively studied. The median age was 34 years (range 18-57 years) and 23 (59%) were male. The majority of patients (80%) had prior autologous SCT. Most (90%) patients received reduced intensity conditioning, 59% received matched sibling donor and 41% matched-unrelated donor grafts.

Results: 36 patients survived beyond 100 days and were evaluable for chronic GVHD of whom 22 (61%) remained free of cGVHD and 14 (39%) developed cGVHD. The disease status at day 100 post-transplant was reported in 36 out of 39 evaluable patients. 7 (19%) achieved a CR, 11 (31%) had a PR, 15 (42%) a stable disease and 3 (8%) had progressive disease. Following transplantation 30 (77%) patients have relapsed or progressed at a median of 12.7 months (range 1-39 months) post-transplant. With a median follow-up of 28 months (range 3-93 months) 7 patients remain alive in complete remission, 2 are in stable disease and 26 have died. The Kaplan-Meier estimates PFS at five years was 18%. 6 patients (18%) died of non-relapse mortality (NRM) at a median of 303 days (range 28 days-40 months) following transplantation. The causes of death included infection (n=2), GVHD (n=3), multi-organ failure (n=1).

Summary/Conclusions: Allogeneic SCT outcomes could be a viable option for patients who are refractory to salvage chemotherapy, especially because better results are obtained when this treatment is applied earlier. Despite the reduction of NRM and GVHD, disease relapse still represents the major issue in the setting of allogeneic SCT failure. The availability of novel agents resulting in objective responses may eventually result in increased eligibility for allogeneic SCT.

E1562

RESULTS OF THE IMPLEMENTATION OF CRYOTHERAPY IN PROTOCOLS OF ORAL MUCOSITIS PROPHYLAXIS IN PATIENTS SUBJECT TO A TRANSPLANT OF HEMATOPOYETIC PROGENITORS. EXPERIENCE OF ONE CENTER

E. Fernández Poveda1,*, V. Cabanas-Pérez2,1, A. Sánchez-Salinas1, M. Blanquer-Blanquer1, M. Berenguer-Piquer2,1, M. Moya-Arna1, A. Martínez-Marin1, J.M. Moraleda-Jiménez1
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Background: Oral mucositis (OM) is one of the main complication during stem cell transplantation (SCT). It has an incidence varies between 47-100%. Numerous prevention strategies have been studied. However, the recommendations of the international guidelines have low evidence to back them up. Cryotherapy is used to reduce OM in conditions that use Melphalan. In our center, we have the cryotherapy implemented in our OM prevention protocol since 2012.

Aims: The main aim is to compare the results in terms of incidence and severity of OM (measured according to World Health Organization scale) in patients in whom cryotherapy was applied and in whom it was not applied as well as the necessity of using morphine and parenteral nutrition. The secondary endpoint is to analyze the occurrence and duration of fever and documentation of infection.

Methods: We used a cohort of patients with plasma cell dyscrasias who underwent autologous stem-cell transplant with conditioning melphalan 200, Busulfan-Melphalan 140 or melphalan 100 in hematodilatation regimen in which cryotherapy was not applied (2007-2011) and another cohort in which was applied (2012-2016). The cryotherapy was implemented. It consists of administering ice poles to the patient who must chew before, during and after the infusion of melphalan. The t-Studen and Chi square method was used to estimate the rates of incidence and the baseline characteristics. The regression logistic method was used to the multivariant and univariant analysis. Hazard ratios and 95% were estimated with the use of logistic regression model.

Results: The baselines characteristics can be seen in table 1. All patients in both groups had OM. In the cryotherapy and non-cryotherapy groups, the distribution was respectively: grade I 20% vs 16%, grade II 40% vs 10.8%, grade III 31.4% vs 59.4% and grade IV 8.5% vs 13.5%. We observed a reduction in the incidence of severe mucositis (grade III and IV) in the group in which cryotherapy was used against the cohort in which it was not (40% vs 72.9%, p=0.005). The need for morphine was also lower in the cryotherapy cohort (84% vs 72%, p=0.149). The use of parenteral nutrition was lower in the non-cryotherapy group (8.57% vs 13.5%, p=0.7). The prevalence of fever was predominant in the cryotherapy group (51% vs 43%, p=0.48), but and infection was documented on more occasions in cryotherapy group (27% vs 81%, p=0.149). The median number of days the patients were discharged from the cryotherapy group was lower (14 vs 15 median days, p=0.39) and the mortality at day 100 was higher in the non-cryotherapy group (0% vs 8%, p=0.24).

Decreased mucositis degree was associated in both univariate and multivariate analysis only with cryotherapy (p= 0.01 and p=0.0003). Hazard ratio was 0.81 (IC 95% 0.06-0.55).

Table 1.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Non-CRYOTHERAPY</th>
<th>CRYOTHERAPY</th>
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<tbody>
<tr>
<td>Gender F/M (%)</td>
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<tr>
<td>Age Median (y)</td>
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<tr>
<td>CR/PR (%)</td>
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<td>VPR (%)</td>
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<tr>
<td>GvHD (%)</td>
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<tr>
<td>Infusion (%)</td>
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<td>Moro (%)</td>
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</table>

Summary/Conclusions: In our center, cryotherapy reduces significantly the severity of mucositis. The use of morphine and parenteral nutrition and other complications do not present such a drastic decline, probably because they influence the gastrointestinal mucositis, which is not combated with cryotherapy. With this results, we are encouraged to continue to include cryotherapy in our protocols.

E1563

REDUCED INCIDENCE OF PRIMARY GRAFT FAILURE IN PATIENTS UNDERGOING HAPLOIDENTICAL STEM CELLS TRANSPLANTATION: A SINGLE CENTER EXPERIENCE

A. Martínez-Velandia1,*, M. Gasoin1, R. de Paz1, S. Cortez1, D. Bueno2, A. Sastre2, M. Canales1, A. Pérez-Martínez2

Background: We examined allogeneic transplantation outcomes patients with HL...
Patients with lymphoma

Aims:

of haploidentical allo-HSCT compared to those of HLA-matched allo-HSCT in alternative sources of stem cells for allo-HSCT

nonmalignant hematologic disorders. However, only about a third of candidates

RESULTS OF HAPLOIDENTIC HEMATOPOIETIC STEM-CELL TRANSPLANTATION IN PATIENTS WITH LYMPHOMA: A SINGLE CENTER EXPERIENCE

Methods: We retrospectively analyzed 40 consecutive patients who underwent HSCT from 2014 to 2016: unmanipulated for 20 adults and graft engineering for 20 children (CD34 selection/CD45RA depletion, n=14). The stem cell source was mobilized peripheral blood in all cases. GCSF was systemically used from day 5 until engraftment. We used descriptive statistical methods for analysis.

Results: Patient characteristics are described in Table 1. Conditioning regimen was Bu-Flu-Cy (n=18, adults), Thio-Bu-Flu (n=2, adults), Flu-Mel-Thio for all pediatric patients. ATG was used in 6 children and TLI in 14 children. All adult patients were given PT-Cy. Only one adult patient had high titer donor specific anti HLA antibodies and was desensitized with plasma exchange, Rituximab and IVIG before transplantation. All patients engrafted before day 28 and no PGF diagnosis was established in our serie. We found that 4 patients (3 children, 1 adult) required a boost of CD34 selected graft from the same donor for secondary GF and poor graft function.

Summary/Conclusions: PGF incidence described in literature is 5-10%, we did not find any primary graft failure in our serie. Desensitization therapy appeared to be effective in one patient with anti HLA antibodies. All CD34 boosts were performed for secondary graft failure/poor graft function due to treatment toxicities or viral infections. Unfortunately, analysis of causes and risk factors for secondary GF requires a larger number of patients to be determined.

E1564

RESULTS OF HAPLOIDENTIC HEMATOPOIETIC STEM-CELL TRANSPLANTATION IN PATIENTS WITH LYMPHOMA: A SINGLE CENTER EXPERIENCE

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Background: Allogeneic hematopoietic stem-cell transplantation (allo-HSCT) is a potentially curative treatment for a variety of hematologic malignancies and nonmalignant hematologic disorders. However, only about a third of candidates for allo-HSCT have HLA-matched siblings. For patients who lack HLA-matched siblings, partially HLA-mismatched (haploidentical) related donors are good alternative sources of stem cells for allo-HSCT.

Aims: In this retrospective, single center study we evaluated safety and efficacy of haploidentical allo-HSCT compared to those of HLA-matched allo-HSCT in patients with lymphoma

Methods: A total of 81 lymphoma patients (Hodgkin and Nonhodgkin) with a mean age of 42 years who underwent allo-HSCT (HLA matched n=46, haploidentic n=35) between July 2010 and July 2016 were analyzed. All patients received Cyclophosphamide (Cy) 50mg/kg i.v. on days +3 and +4. All patients initiated CsA day +5, and then adjusted according to the plasma levels. In addition to CsA, all haploidentical allo-HSCT recipients received MMF until day +35.

Results: There were no significant differences in age, sex, diagnosis, disease status up-front HSCT, or transplant characteristics between the groups except a higher median number of stem cells infused in haploidentical group (p=0.004). The median follow-up was 13 months for haploidentical group and 12 months for HLA-matched group. Outcomes of patients are summarized in Table 1.

Summary/Conclusions: Our results suggest that haploidentical allo-HSCT is a safe treatment modality in patients with relapsed lymphoma who lack HLA-matched siblings. The major problem are seems to be viral infections. Future challenges remain in improving post-transplant immune reconstitution and finding the best approach to reduce the incidence and severity of viral infections, while preserving graft-versus-lymphoma effect to prevent the recurrence of the underlying disease.

E1565

COLLECTION OF PERIPHERAL BLOOD HEMATOPOIETIC PROGENITOR CELLS (PBPC) FROM HEALTHY DONORS: 15 YEARS SINGLE CENTER EXPERIENCE

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Background: hematopoietic stem cell transplantation (HCT) is, nowadays, a consolidated therapy within the treatment of multiple hematological pathologies. In the last two decades, the main method of obtaining hematopoietic progenitor cells is blood leukapheresis after mobilization with granulocytic colony growth factors (G-CSF).

Aims: To describe the experience of our center in apheresis of healthy family donors in the last 15 years. Furthermore, analyze the influence of different variables on the procedure and the yields obtained.

Methods: retrospective analysis was performed on 189 hematopoietic progenitor cell collection (HPCC) from January 2002 to December 2016. The study was carried out at Apheresis Unit, Hospital de La Princesa, Madrid, Spain. Progenitor cells mobilization was performed with G-CSF in all cases at a dose of 10mg/kg b.w. Apheresis device was COBE Spectra in all cases and citrate was the anticoagulant used for all the apheresis procedures. All donors were carefully evaluated and informed on the donation procedure and signed an informed consent for apheresis. The venous access used was mostly peripheral venous access in antecubital veins, and in only 7 cases (3.7%) central venous catheter was required. Donor details studied were age, sex, AB0 group, number of apheresis, number of CD34+ per kilogram collected, and processed volume.

Results: among the 189 donors, 85 were females and 104 were males (45% vs 55%). The hematologic pathologies that motivated transplantation were, in order of frequency, Acute Myeloid Leukemia (AML) (40.2%), Myelodysplastic Syndrome (MDS) (13.8%), Acute Lymphoblastic Leukemia (ALL) (10.1%), Hodgkin’s Lymphoma (HL) (8.5%), Non-Hodgkin’s Lymphoma (NHL) (6.3%), Multiple Myeloma (MM) (5.3%), Chronic Myeloid Leukemia (CML) (4.2%), other (11.6%). Apheresis donors most of young AB0 group, and majority of cases (65%) donor and recipient had the same group. Median weight of donors was 74 Kg and in recipients was 70.5 Kg. Median age of our donors was 50 and median age of recipients was 51 years. Twenty donors were >65 years (10.6%) and 10 were >70 years (5.3%). Median of processed volume was 13 liters, but if we stratify that volume by recipient’s weight, in those whose were heavier than 100 kg, median of processed volume was 18 liters. Two apheresis procedures were performed only in ten donors. Of these, 2 were older than 70 years (20% of total donors over 70 years of age) compared to 8 under 70 years of age (4.5% of all patients in that age range). The median of CD34+ /kg collected was 5 x 106. Among the age ranges, median yield of CD34+ in patients older than 70 years was 3.55 x 106, in patients between 31 and 69 years was 4.96 x 106 and in patients younger than 30 years was 5.5 x 106. The apheresis procedure was mostly well tolerated, with only mild symptoms of hypocalcemia and disturbances related to venous access in a minority of cases. No significant long term adverse effect has been observed in the blood tests reported to our centers during the five years of follow up after the donation.

Summary/Conclusions: donor age and weight discrepancy with recipient were the factors that significantly affected PBPC yields in our experience in healthy donors. These factors had also an impact in the amount of liters of volemia processed, although in most cases only one apheresis procedure was enough. Adverse effects of apheresis for PBPC collection were the same as for other apheresis procedures such as those related to venous access, almost always peripheral one and citrate toxicity.

haematologica | 2017; 102(s2) | 637
E1566
ALLORESPONSES OF HUMAN T-CELLS FROM ADULT PERIPHERAL BLOOD AND UMBILICAL CORD BLOOD ARE DIFFERENTIALLY IMPACTED BY LENALIDOMIDE - IMPLICATIONS FOR AHSCT
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Background: Immunomodulatory drugs (IMiDs), such as lenalidomide provide a tool to enhance both direct anti-tumor and graft-versus-host effects after allogeneic haematopoietic stem-cell transplantation (AH SCT). However, early clinical experience with IMiDs after AHSCT using adult peripheral blood (APB) as a stem cell source has been limited by induction of graft-versus-host disease. Characterization of the mechanisms by which IMiDs can modulate alloresponses of T-cells from different cell sources could facilitate more effective use of these drugs in the setting of AHSCT.
Aims: To use in vitro modelling to identify changes in alloresponses of APB and umbilical cord blood (UCB) T-cells after exposure to the widely used IMID lenalidomide.

Methods: We have used multi-parameter flow cytometry and gene expression profiling to perform an in-depth characterisation of the phenotypic and genotypic effects of clinically relevant concentrations of lenalidomide treatment on T-cells during allogeneic co-culture. Using GCSF-mobilised APB (GM PB), steady state APB and UCB PBMC as responder cells in allogeneic co-culture we have been able to compare the differential effect of lenalidomide on these three cell sources. Allogeneic responder cells were labelled with CFSE (carboxyfluorescein diacetate succinimidyl ester) to allow quantification of allo-proliferation. Responder T-cell subsets including naive, memory, activated, cytotoxic and regulatory were interrogated. Functional effects of lenalidomide treatment including cellular capacity to produce cytokines, degranulate and exert direct cytotoxicity was also assessed. RNA was extracted from highly purified proliferative and non-proliferative CD8+ T-cell fractions following a combination of magnetic and flow-sorting and gene expression changes assessed by Affymetrix whole genome array and qRT-PCR.

Results: We demonstrate that lenalidomide increases net alloproliferation of APB T-cells by selectively enhancing allospecific proliferation of CD8* T-cells. These CD8* T-cells have enhanced effector memory differentiation, are enriched for polyfunctional effectors, and have a distinct gene expression profile with altered expression of key immunoregulatory genes and pathways. This effect on CD8* T-cell proliferation was seen across all 3 cell sources. Importantly a differential effect on CD4* T-cell responses was observed depending on cell source. Lenalidomide treatment of APB results in no change in CD4* T-cell proliferation overall, but leads to reduced frequencies of CD4* regulatory T-cells (Treg). In contrast lenalidomide treatment of GMPB resulted in a significant increase in CD4* T-cell proliferation, with no effect on Treg cell frequencies. Most strikingly, although lenalidomide treatment of UCB T-cells during allosimulation results in a similar increase in alloreactive effector CD8*T-cells, it also reduces allospecific proliferation of CD4*T-cells and selectively expands frequencies of Treg, resulting in a net reduction in UCB T-cell alloproliferation.
Summary/Conclusions: Our findings show that lenalidomide has a qualitatively different impact on alloresponses of T-cells from different cell sources, with a potentially tolerogenic effect on UCB T-cells. These findings have important implications for the future use of IMiDs in the setting of AHSCT.

Figure 1.

E1567
USING MARKER GENES ANALYSIS INSTEAD OF MLR ASSAY FOR IDENTIFICATION OF FUNCTIONAL CD4+FOXP3+ REGULATORY T CELLS IN GVHD PROPHYLAXIS
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Background: There are two types of CD4+CD25+FoxP3+ regulatory T cells (Treg), natural Treg cells (nTreg): developing in thymus, and induced Treg cells (iTreg) arising from CD4+ naïve T cells. The iTreg cells have been considered important for maintenance of immunological tolerance and correlate with the occurrence of GVHD in some studies. Establishing a quick method to identify the functional iTreg cells is worthy of focusing. Five to ten percent Tregs could be found in human CD4+ T cells and should be expanded via in vitro culture. In order to improve the efficiency of Treg cells for the prevention of GVHD, we attempt to establish a relatively quick analytic method to identify the functional iTreg cells, and then to curtail the iTreg cells harvest time for clinical use. Therefore, using qPCR for marker genes analysis instead of MLR (mixed lymphocyte reaction) assay is an important issue.
Methods: Mouse splenocytes were prepared from mouse spleen. Human PBSC were prepared from peripheral blood (PB) of healthy donors by Ficoll-Hypaque density gradient centrifugation. All T cells were isolated by negative selection, then CD4+ naïve T cells were harvested. CD4+ naïve T cells were activated with anti-CD3/CD28 beads in the presence of IL-2, TGF-β and retinoic acid (RA) containing RPMI1640 medium. The protocol is showed in Fig. 1.

Results: Seven genes for qPCR analysis were used to identify the functional iTreg cells. We used the different proportions of iTreg cells in total naïve T cells for 7 genes expression analysis and MLR assay to investigate the relationship between gene expression and functional iTreg cell frequency. Treg cells with altered gene expression were identified and the data of marker genes expression of iTreg cells were shown in Table 1. Then the iTreg cell populations were sorted and qPCR analysis was performed. The results showed that iTreg cells with altered marker genes expression could be identified and sorted. The marker genes expression of iTreg cells was shown in Fig. 2. The results indicated that the expression profile of iTreg cells was different from naïve T cells.

Figure 1.

Methods: We have used multi-parameter flow cytometry and gene expression profiling to perform an in-depth characterisation of the phenotypic and genotypic effects of clinically relevant concentrations of lenalidomide treatment on T-cells during allogeneic co-culture. Using GCSF-mobilised APB (GM PB), steady state APB and UCB PBMC as responder cells in allogeneic co-culture we have been able to compare the differential effect of lenalidomide on these three cell sources. Allogeneic responder cells were labelled with CFSE (carboxyfluorescein diacetate succinimidyl ester) to allow quantification of allo-proliferation. Responder T-cell subsets including naive, memory, activated, cytotoxic and regulatory were interrogated. Functional effects of lenalidomide treatment including cellular capacity to produce cytokines, degranulate and exert direct cytotoxicity was also assessed. RNA was extracted from highly purified proliferative and non-proliferative CD8+ T-cell fractions following a combination of magnetic and flow-sorting and gene expression changes assessed by Affymetrix whole genome array and qRT-PCR.
experiments, iTreg cells induction could be TGF-b1 dependent. After different amount of TGF-b1 induction, the genes expression profile also showed the coincidence of the data in Fig.2 (Fig.3). Using the same iTreg populations, MLR assay have been investigated for 5 days. The T cell suppression percentage would be dependent on the iTreg cells proportion (Fig.4A and B). It indicated that the gene expression levels can represent the biological function of iTreg cells. It’s the better way to identify the iTreg cells. Further, we have used PBMCs for Treg cell induction, the marker genes expression analysis also showed in Fig.5. After comparing with IL-2 cultured T cells, the gene expressions revealed the difference in between iTreg cells and un-induced T cells.

Summary/Conclusions: Our study showed that MLR assay should spend 3 to 5 days for identification of the functional iTreg cells, however, the marker genes analysis took only one day for that. Besides, MLR assay is a more complicated method than qPCR analysis. Using simple analysis for human iTreg cells functional identification could save the time for clinical application and might prevent GVHD occurrence effectively.

E1568

OXIDANT-ANTIOXIDANT SYSTEM IN PATIENTS WITH MULTIPLE MYELOMA
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Background: Multiple myeloma (MM) is one of the most widespread malignant B-cell lymphoproliferative disorders and is characterized by a clonal proliferation of atypical plasma cells in bone marrow or, less frequently, in extramedullary locations synthesizing monoclonal immunoglobulins. Currently, autologous hematopoietic stem cell transplantation (auto-HSCT) is recognized as the standard method of treatment for young patients (<65 years old) with MM. Moreover, the best auto-HSCT results are observed in patients who have received new medication (thalidomide, bortezomib, and lenalidomide) during induction therapy and who have achieved at least a very good partial response, which leads to a significant increase in overall survival. However, studies reflecting the impact of this kind of treatment on the dynamics of oxidant-antioxidant indicators are virtually non-existent. At the same time, the possibility of treating developing diseases by prescribing medication makes the problem highly relevant.

Aims: The aim of the study was to investigate the state of OS-AOS in patients with MM during auto-HSCT.

Methods: We studied 20 patients (11 men and 9 women, mean age 49 years) who followed auto-HSCT after high-dose melphalan. The control group consisted of 50 age- and sex-matched healthy persons. The plasma levels of malonic dialdehyde and ceruloplasmin as well as activities of superoxide dismutase and catalase were measured by standard biochemical techniques. In erythrocytes, the level of non-protein thiol groups was studied. The state of OS-AOS was investigated in each patient four times: before and after conditioning with melphalan, at the maximal moment leukocyte decrease and after complete reconstitution from cytopenia.

Results: We have found the features of impaired balance in OS-AOS in MM patients before as well as in course of auto-HSCT. The level of malonic dialdehyde in MM patients was not significantly different from that in the control group. At the same time, ceruloplasmin plasma level as well as catalase activity were significantly increased in patient group (p<0.05), whereas the level of non-protein thiol groups was decreased in MM (p<0.05). The results of our study have shown, that an imbalance of OS-AOS is frequently seen in MM patients and, possibly, could influence the course of auto-HSCT.

Summary/Conclusions: The results of the study indicate a high frequency of occurrence of disturbance of the condition of OS-AOS in patients with MM. The imbalance in the functioning of this system is not entirely eliminated in the process of treating the patients with MM using auto-HSCT. The question of the necessity and methods of the possible correction of OS-AOS in patients with MM, particularly during auto-HSCT, requires further study.

E1569

SURFACE RECEPTOR EXPRESSION PROFILE DEFINES ALLOREACTIVE DONOR CD8+ T-CELLS AFTER MURINE ALLOGENIC HEMATOPOIETIC CELL TRANSPLANTATION
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Background: Acute graft-versus-host disease (aGVHD) is a severe and often life-threatening inflammatory complication of allogeneic hematopoietic cell transplantation (allo-HCT). aGVHD is mediated by alloreactive donor T cells attacking the gastrointestinal tract, liver, and skin of the host. Efficient strategies to improve aGVHD-related morbidity and mortality will rely on more precise methods than preemptive immunosuppression to consistently predict aGVHD and abrogate disease manifestation without exposing patients to an unwarranted risk for infectious complications. Recent insights into the multistep-pathophysiology of aGVHD provide a good basis for the development of new tests to identify individual patients at risk before the onset of aGVHD.

Aims: As pathologic T cell responses rely on spatiotemporally defined programs of T cell activation, acquisition of effector functions, and homing to GVHD target tissues it appeared attractive to assess receptor expression profiles of peripheral blood T cells as potential predictive markers.

Methods: Therefore, we characterized the surface receptor expression profile of peripheral blood donor lymphocytes early after allo-HCT in two independent murine models across minor histocompatibility antigens (miHAg) with multicolor flow cytometry. C57Bl/6 (H-2b, Thy1.1+) or B10.D2 (H-2d, Thy1.1+) T cells plus bone marrow cells were transplanted in conditioned (8Gy) miHAg mismatched BALB/c (H-2b, Thy1.2+) and syngeneic C57Bl/6 (9Gy) or BALB/c (H-2d, Thy1.1+) recipients. To identify suitable predictive markers, we compared the expression pattern of allo-HCT recipients to syngeneic HCT recipients and untreated wild type controls.

Results: Comparing a panel of T cell surface receptors, we found the homing markers 4937 integrin, and P- and E-selectin ligand highly up-regulated on allogeneic peripheral blood donor CD8+ T cells at peak time points of cell migration. The combination of these homing markers with the activation markers CD25 and CD69 at later time points and low expression levels of L-selectin allowed to define alloreactive donor T cells.

Summary/Conclusions: Based on this data we propose that alloreactive CD8+ T cells can be identified in miHAg allo-HCT recipients upon their homing receptor expression pattern as soon as six to ten days before the onset of aGVHD.
**E1570**

**SOLUBLE FORM OF TRANSFERRIN RECEPTOR IS ASSOCIATED WITH AGE AT DIAGNOSIS AND RISK OF THERAPEUTICAL INTERVENTION AND IRON OVERLOAD IN PATIENTS WITH NON-TRANSFUSION-DEPENDENT THALASSEMIA**

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**Background:** The soluble transferrin receptor (sTfR), that fully reflects the narrow erythropoietic activity, was found to have not only a striking diagnostic accuracy in predicting the risk of extramedullary haematopoiesis (EMH), but also in scoring disease severity in non-transfusion-dependent thalassemias (NTDT).

**Aims:** We retrospectively evaluated the relationship between sTfR and some fundamental events in the life and in the management of patients with NTDT.

**Methods:** We considered 111 NTDT patients with four genetic entities of NTDT: homozygous or compound heterozygous state for β-thalassemia, triplicated a genotype associated with β heterozygosity, deletional HbH, and combination of a β defect plus a β chain variant. sTfR was measured with a commercially available kit. A group of patients was enrolled in the Myocardial Iron Overload in Thalassaemia (MIOT) network and underwent hepatic iron overload assessment by the T2* Magnetic resonance Imaging (MRI) technique.

**Results:** The group with homozygous or compound heterozygous for β-thalassemia had the higher sTfR levels. sTfR values were negatively related to age at diagnosis (R=-0.462, P<0.0001), and to age at first transfusion (R=-0.703, P<0.0001). At ROC curve a sTfR=5.3mg/L discriminated the patients with a previous history of occasional transfusions. sTfR values were significantly higher in splenectomized patients. sTfR values were negatively related to age at splenectomy (R=-0.328, P=0.044) and in unsplenectomized patients a significant positive correlation was found between sTfR values and spleen diameter (R=0.572, P<0.0001). sTfR values were negatively related to age at starting chelation therapy (R=-0.564, P=0.044). Patients never chelated showed significantly lower sTfR values than patients under chelation therapy (see Figure). sTfR values were significantly correlated with serum ferritin levels (R=0.321, P<0.0001), but no with LIC values.

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**E1571**

**LOW SERUM FERRITIN LEVELS DO NOT PROTECT FROM CARDIAC AND HEPATIC IRON IN PATIENTS WITH THALASSEMIA MAJOR**

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**Background:** The heterogeneity of patients with NTDT is an emerging cause of complex management and treatment of the disease. Our data indicate that the measurement of sTfR level, a common laboratory test, could contribute to correctly stratify the disease history and the chelation strategy in NTDT.

**Aims:** To measure the levels of IMA in 45 children and adolescents with β-TM compared with 30 healthy controls and assess its relation to lipid peroxidation, vascular complications and subclinical atherosclerosis.

**Methods:** β-TM patients without symptoms of heart disease were studied focusing on transfusion history, chelation therapy, serum ferritin, malondialdehyde (MDA) and IMA levels. Echocardiography was performed and carotid intima media thickness (IMT) was assessed.

**Results:** IMA and MDA levels were significantly higher in β-TM patients compared with controls (p<0.001). IMA was higher among patients with heart disease and pulmonary hypertension (PH) risk than those without. Serum IMA and MDA levels were elevated among patients with serum ferritin ≥2500 µg/L compared with patients below this cutoff. TM patients compliant to chelation had a significantly lower IMA levels than non-compliant ones. Receiver operating characteristic (ROC) curve analysis revealed that a cutoff value of IMA at 75 U/mL could differentiate β-TM patients with PH risk with 90% sensitivity, compared with 30 healthy controls and assess its relation to lipid peroxidation, vascular complications and subclinical atherosclerosis.

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91.4% specificity and positive predictive value of 75% and negative predictive value 97%; area under the curve 0.883 (95% confidence interval 0.752-0.959). In addition, the cutoff value of IMA at 17.5 U/ml could differentiate β-TM patients with heart disease with 80.5% sensitivity, 88.9% specificity and positive predictive value of 96.7% and negative predictive value 73.3%; area under the curve 0.887 (95% confidence interval 0.750-0.962). Significant positive correlations were found between IMA levels and disease duration (r=0.311, p=0.045), white blood cell count (r=0.322, p=0.031), serum alanine aminotransferase (r=0.388, p<0.01) and aspartate aminotransferase (r=0.382, p=0.037). IMA and MDA levels were positively correlated (r=0.503, p<0.001) and there was a significant positive correlation between these two markers and mean serum ferritin (IMA; r=0.945, p<0.001 and MDA; r=0.957, p<0.001) among TM patients. IMA levels were positively correlated to TRV (r=0.621, p<0.008) while negatively correlated to ejection fraction (r=0.412, p=0.014) and fractional shortening. Both IMA and MDA were positively correlated to CIMP (r=0.607, p<0.001 and r=0.516, p=0.001, respectively).

Summary/Conclusions: Our results highlight the role of oxidative stress in the pathophysiology of vascular complications in thalassemia. IMA could be useful for screening of β-TM patients at risk of cardiopulmonary complications and atherosclerosis because its alteration occurs in early subclinical disease.

E1573
SERUM N-TERMINAL PRO-BRAIN NATRIURETIC PEPTIDE LEVEL AND ECHOCARDIOGRAPHIC TISSUE DOPPLER ABNORMALITIES IN PATIENTS WITH BETTA THALASSEMIA MAJOR
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Background: Heart disease remains the major cause of morbidity and mortality in thalassemia patients. Multiple pathologies have been implicated in the development of cardiac dysfunction in these patients including: cardiac iron overload leading to right ventricular diastolic then left ventricular systolic dysfunction, chronic anemia and tissue hypoxia. Because congestive heart failure is the main cause of death in these patients, early recognition of cardiac dysfunction may be useful in modifying therapy in a timely manner. Tissue Doppler imaging (TDI) is a technique that allows measurement of cardiac velocities using tissue Doppler imaging. Serum brain natriuretic peptide (BNP) level may be promising tools for such a purpose.

Aims: This study aimed to assess serum NT-proBNP level and echocardiographic tissue doppler abnormalities among a cohort of Egyptian beta thalassemia major patients and to detect possible associations between them as well as other disease variables including iron overload.

Methods: Thirty beta thalassemia major patients with a mean age of 12.93±2.07 years regularly followed up at Pediatric Hematology Clinic, Cairo University and thirty aged matched healthy control subjects were included. Conventional, M-Mode and TDI echocardiography were performed to all patients and control subjects in addition to cardiac magnetic resonance (CMR) for studied patients. Serum NT-proBNP level was measured using enzyme linked immunosorbant assay (ELISA).

Results: Tissue doppler imaging revealed a significant difference of ratio of the early (e') to late (a') right ventricular filling velocities (Rv e'/a' ratio) between cardiac iron overloaded patients reflecting early diastolic dysfunction in cardiac iron overloaded patients. Myocardial performance index of left ventricle (LV_TEI index) by TDI showed significant difference in cardiac iron overloaded patients compared to non cardiac iron overloaded patient (mean v=0.54±0.04 with p value=0.003) indicating decrease in ventricular relaxation due to iron overload and restrictive cardiomyopathy. SerumBNP level was significantly higher among patients compared to controls (mean 99.18±72.43pg/ml versus 18.93±9.65pg/ml respectively with p value<0.001) and among cardiac iron overloaded patients compared to non cardiac iron overloaded (mean 212.5±57.18pg/ml versus 64.75±26.69pg/ml respectively with p value<0.001). We found positive correlation between level of BNP and frequency of the blood transfusion/year, Rv/e'a and LV_TEI_TDI index with (p value 0.006, <0.001 and 0.030 respectively) denoting early diastolic impairment in asymptomatic thalassemia patients.

Summary/Conclusions: Asymptomatic thalassemia major patients under chelation therapy may have diastolic and or systolic dysfunctions that could not be detected by conventional echocardiography but could be highlighted by TDI. CMR, TDI and serum BNP level measurement are promising tools for accurate assessment of cardiac functions and iron overload in thalassemia patients.

E1574
PRENATAL DIAGNOSIS OF HEMOGLOBINOPATHIES IN NORTHERN GREECE. 15 YEARS REPORT
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Background: Hemoglobinopathies constitute the most frequent monogenic disorders worldwide and thalassemias are the most frequent genetic disorders in the Mediterranean basin. The disease frequency of thalassemia is 1:50, while 15% of the population are carriers of the Hb S mutation. The rate of β-thal carriers could be as high as 15-20% in some areas. The risk of giving birth to an affected child depends on the incidence of the thalassemic gene and this may vary from 1/24 to 1/150 in married couples. The National Program for prevention of Thalassemia was established in 1973. Through population screening and prenatal diagnosis programs Greeks and immigrants are screened and counseled.

Aims: We report our findings on prenatal diagnosis of thalassemias and hemoglobinopathies in Northern Greece over a 15 year period (2001-2015).

Methods: During the 15 year period, a total of 33,837 subjects were screened for β-thalassemia and as couples or as individuals. We found 3,598 1 Carrier couples and 3,659 couples screened for hemoglobinopathies. In 371 couples both partners carried an abnormal Hb gene and counseling was offered and 329 pregnancies were found at risk of giving birth to an affected child. The genetic interactions were in 245 pregnancies at risk for thalassemia major offsprings and 84 for sickle cell disease ones. Prenatal diagnosis was offered in all 12 weeks of gestation (n=298), in few cases by amniotic fluid sampling (n= 21) collected at 16-18 weeks. Few late counsels were tested by fetal blood sampling at 20 week of gestation(n=5). The remaining 42 pregnancies involved couples who were double heterozygotes for mutations that did not cause severe clinical disease and were exempted from prenatal diagnosis. The gene interactions were as follows β-thal /α-thal, β-thal in combination with Hb E-Saskatoon or D-Punjab, Hb E/HbE, Hb E-Saskatoon /with carrier of Hbs, and Hb O/ Hb O, β-thal or α-thal in combination with D Punjab, Hb Brugg/β-thal, silent β-thal/ silent β-thal. 91% of the couples were of Greek origin, and 9% were immigrants from Asia Minor, Turkey, Albanian, Somali, Nigeria, Fyrom, Ruanta and Thailand. We had an average of 15-32 prenatal diagnosis per year.

Results: The results of DNA analyses of the samples were as follows: 76 fetuses (23%) were found to be homozygote or double heterozygote for clinical significant mutations. These couples were informed of the danger of having an affected child but the termination or continuation of the pregnancy was left to the couples to decide. Nevertheless all, except three couples, preferred to terminate the pregnancies so we had one case of thalassemia major offspring and two cases of silent β-thal/ O Arab offsprings born. Selective abortion of the affected fetus was performed in the cases of the twin pregnancies (n=6). There have been no cases of misdiagnosed pregnancies and only one obstetric complication (rupture of membrane that lead to miscarriage) was reported.

Summary/Conclusions: It is universally accepted that thalassemia prevention programs are successful in countries with a high frequency of Hb mutations, and prenatal diagnosis is mandatory in all at risk couples. The National Thalassemia Prevention Program has effectively decreased the incidence of thalassemia major and sickle cell syndromes in our country and in our region.

E1575
THE IMPACT OF LIVER STEATOSIS ON THE ABILITY OF SERUM FERRITIN LEVELS TO PREDICT LIVER IRON CONCENTRATION AMONG THE IMPACT OF LIVER STEATOSIS ON THE ABILITY OF SERUM FERRITIN LEVELS TO PREDICT LIVER IRON CONCENTRATION AMONG NON-TURFUSION-DEPENDENT THALASSAEMIA PATIENTS: A CROSS-SECTIONAL EVALUATION
P. Ricchi1, A. Meloni2, L. Pistoia2, V. Posilano2, P. Preziosi3, A. Filosa1, A. Pepe2

Background: Fatty liver is a common abnormality encountered in western countries among patients undergoing imaging of the abdomen and is associated to systemic inflammation and to increased ferritin levels, frequently unrelated to iron overload.

Aims: We analyzed the impact of the presence of fatty liver in the parameters of iron overload among our patients with Non Transfusion dependent Thalassemia (NTTD).

Methods: 111 patients with NTD present. The diagnosis was made by means of abdominal ultrasound (US), liver biopsy or magnetic resonance imaging. Liver steatosis was classified as mild (0-25%) moderate (25-74%) or severe (≥75%). Serum ferritin, liver enzymes, ALT/AST ratio and iron overload parameters (serum and hepatic ferritin, serum and liver transferrin receptor index, serum and liver α1 antitrypsin/haptoglobin 1 assay ratio) were measured. The results were analyzed using Student’s t-test and Kruskal-Wallis test.

Results: Liver steatosis was frequently (35.5%) encountered among our patients with NTD and was significantly more prevalent in males with respect to females (49.0% vs 24.6%, p<0.008). Patients with liver steatosis had significantly higher levels of ALT, AST, ALT/AST ratio and ferritins than those without, but LIC values were comparable (Table 1). At ROC curve analysis, an ALT/AST >0.89 predicted the presence of liver steatosis with a sensitivity=0.872 and a specificity=0.901 (P<0.0001). Overall, ferritin levels positively correlated with LIC values (R=0.558, P<0.0001) but in patients without steatosis there...
was a strong relationship between ferritin and LIC values (R=0.656, P<0.0001) while in patients with steatosis the correlation was moderate (R=0.428, P=0.05).

| Table 1. |

**Summary/Conclusions:** Our data show that liver steatosis affected also patients with NTDT and should be suspected in presence of a ALT/AST ratio >0.89. Recently, serum ferritin thresholds to predict clinically relevant liver iron concentrations for guiding chelation therapy when MRI is unavailable in patients with (NTDT) have been provided. Our data show that the presence of liver steatosis may lead to overestimate the magnitude of iron burden and may be responsible for anticipating or exceeding chelation treatment in patients with NTDT in absence of a LIC evaluation.

**E1576**

**CIRCULATING CELL-FREE DNA (CFDNA) AND INEFFECTIVEERYTHROPOIESIS IN THALASSEMIA INTERMEDIA**

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**Background:** Low concentrations of circulating cell-free DNA (cfdNA) are found in the plasma of healthy individuals and increase in a number of conditions, to clinical severity, including cancer, chronic inflammation, autoimmune diseases and trauma. The mechanisms of release of cdNA in the bloodstream are not well understood: DNA could originate from cells undergoing apoptosis/necrosis in tissues or from cells released in the blood and subsequently lysed. Also the tissue origin of cdNA is mainly unclear. It has been suggested that cdNA, at least after bone-marrow transplantation, could be mostly of hematopoietic origin. This finding prompted us to explore whether cdNA is increased in patients with ineffective erythropoiesis (IE), a condition characterized by the over-proliferation and lysis/removal of erythroid precursors. This situation is common in thalassemias, mainly in non transfusion-dependent patients (NTDT).

**Aims:** The present study was designed i) to evaluate the behaviour of cdNA in IE caused by beta-thalassemia, and ii) to assess whether cdNA could be useful to quantify IE.

**Methods:** We studied 49 beta-thalassemia intermedia (TI) patients (mean age 41 years, range 16-65), 23 of whom were splenectomized. No evidences of tumor, trauma or autoimmune diseases have been observed in any patient at time of the study. Eighteen healthy subjects were also included as control group. The study was approved by the local ethical committee. DNA was extracted by QIAgen silica-based micro-spin columns from 200 mL of K2EDTA plasma. cdDNA concentration determined fluorometrically using the fluorescent dye PicoGreen. Biochemical and hematologic parameters were determined in all patients as a part of laboratory routine. Reticulocytes and peripheral erythroblasts (EBL) were counted by automated procedures. Soluble transferrin receptor (sTfR) and growth differentiation factor 15 (GDF15) were also measured by immunometric ELISA assays.

**Results:** In the 49 patients studied, plasma cfdNA concentrations ranged from 6.3 to 93.1 ng/mL and are significantly higher than in controls (median 21.8 vs 10.4, <.0001). Comparing non splenectomized (n-SPX) patients, we observed a significant increase of cfdNA in the SPX group (median 29.4 vs 19.3 ng/mL, p=0.0085). In the whole TI group, cfdNA concentration was significantly correlated with EBL (p<.0001), LDH (r=0.52, p=0.001) and AST (r=0.56, p<.0001). Correlations of cfdNA were also observed with sTfR (r=0.45, p=0.0014) and GDF15 (r=0.56, p<0.001). Notably, correlations with EBL (r=0.75, p<.0001), AST (r=0.48, p<0.006) and uncorrected bilirubin (r=0.54, P=0.0083) were observed only within the SPX group and not in non-SPX. In patients with SPX, the concentration of cfdNA was significantly higher than in non-SPX (Median 45.9 vs 26.3, p<0.001). In the SPX group, correlations of cfdNA were also found with the amount of IE based on high number of EBL and the lysis of circulating erythroblasts (both increased after splenectomy). We obtained preliminary evidences that circulating cfdNA concentration may be a suitable indicator of erythropoietic activity in TI patients. Results need to be extended on larger samples of patients’ population to investigate the possible use of plasma cfdNA as a feasible and reliable biomarker to describe/monitor the severity of IE and TI complications.

**E1577**

**LEFT VENTRICULAR HYPERTRABECULATION BY CARDIAC MAGNETIC RESONANCE IN THALASSEMIA INTERMEDIA PATIENTS: FREQUENCY AND PROGNOSTIC ROLE**

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**Background:** Differentiation of left ventricle non-compactation (LVNC) from hypertrabeculated LV due to a negative heart remodeling in thalassemia intermedia (TI) can depend on the selected CMR criterion. The recently proposed Piga’s criterion (NC/C ratio threshold of >2.5, Am J Haem 2012) seems to have a low specificity to identify the true LVNC in TI. Anyway, the Piga’s criterion could easily detect a negative heart remodeling in TI patients.

**Aims:** The aim of our study was to prospectively assess whether the Piga’s criterion had a prognostic role for adverse cardiovascular outcomes in TI patients.

**Methods:** We studied prospectively 168 TI patients (81 males, mean age 38.32 ±11.61 years) consecutively enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) Network. Standard cine steady-state free precession sequences were acquired and used for the calculation of biventricular function parameters (short-axis) and for the calculation of the thickness of the non-compact and the compacted myocardium (three diastolic long-axis views) in all 16 segments. The maximal NC/C ratio was considered. Late gadolinium enhancement (LGE) images were acquired to detect myocardial fibrosis.

**Figure 1.**

**Results:** Eight patients were excluded because a cardiac complication was present at the first CMR. The baseline mean age of the considered 161 TI patients was 38.32±11.61 years and 81 patients were males. The study population was divided into two groups: patients with Piga’s positive criterion (n=15, 9.31%) and with Piga’s negative criterion (n=146, 90.68%). No significant differences were found between the two groups in terms of demographic features and CMR parameters. The mean follow-up time was 57.50±21.87 months. Sixteen new cardiac events were recorded: 1 heart failure, 10 supraventricular arrhythmias and 5 pulmonary hypertension. Due to numerical reasons, it was possible to perform a Cox regression analysis only for arrhythmias and cardiac complications globally considered. Patients with Piga’s positive criterion had a significant higher risk of developing arrhythmias (hazard ratio=HR=7.19, 95% CI=2.02-25.51; P<0.002) and cardiac complications (HR=3.86, 95% CI=1.88-11.36; P=0.025). The figure shows the Kaplan-Meier survival curves. The Piga’s positive criterion remained a significant prognosticator also in a multivariate models including previous and resolved events (14 cardiac complications, of which 7 arrhythmias) (HR for arrhythmias=23.67; HR for cardiac complications=7.09).

**Summary/Conclusions:** Based on our data a NC/C ratio >2.5 provides prognostic information for patients with TI about the risk of developing cardiac complications.

**E1578**

**NITRIC OXIDE DYSREGULATION IN BETA-THALASSEMA MAJOR: RELATION TO PULMONARY HYPERTENSION**

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**Background:** Pulmonary hypertension (PH) is emerging as one of the most devastating complications of beta-thalassemia major. Chronic hemolysis and iron overload constitute a major source of strong oxidative stress. Free heme radicals and red cell membrane elements resulting from hemolysis have a negative effect on the intrinsic nitric oxide (NO) production and arginase bioavail-
ability. Deficiency of both biochemical mediators promotes vasoconstriction of the pulmonary vasculature resulting in further endothelial dysfunction, with subsequent intensified reduction of nitric oxide. The role of nitric oxide dysregulation is well-studied in non-transfusion dependent thalassemias and in sickle cell disease, but yet not very well-characterized in beta thalassemia major.
Aims: The aim of our work is to study the relation between intrinsic nitric oxide level and the evolution of pulmonary hypertension in beta thalassemia major.
Methods: This is a case-control study, including all patients with beta thalassemia major above 12 years of age, undergoing follow up in pediatric hematology and in medical research institute, University of Alexandria, Egypt throughout a period of 6 months from 1st of July till 31st of December 2016. All patients were screened for pulmonary hypertension by echocardiography, and those who have high tricuspid regurgitant jet velocity (TRJ V >2.5m/sec.) underwent cardiac catheterization.
Results: The present study included 52 thalassemic patients, 28 males and 24 females aged between 11 and 26 years. The patients were subdivided into two groups (17 patients with pulmonary hypertension (PH), proven by cardiac catheterization and 35 patients without pulmonary hypertension). Nitric oxide level (measured by ELISA) was significantly lower in patients as a whole compared to controls [median of 19 micromol/L versus 30 micromol/L (P=0.02)]. Similarly, nitric oxide was significantly lower in PH group compared to non-PH patients (p=0.001). In addition, there was a statistically significant negative correlation between serum NO level and serum ferritin level in all patients (r=−0.444, p<0.001).
Summary/Conclusions: In conclusion, NO reduction might contribute significantly to the development of pulmonary hypertension in patients with beta thalassemia major. This effect could be related to the degree of hemolysis, iron overload and the duration of disease. Further studies on the adverse pathophysiologic effects of nitric oxide deficiency in beta thalassemia major e.g. its relation to coagulopathy and platelet aggregation are recommended.

E1579
Abstract withdrawn.

E1580
SPECKLE-TRACKING ECHOCARDIOGRAPHY FOR DIAGNOSIS OF EARLY MYOCARDIAL DISEASE IN EGYPTIAN BETA THALASSEMIA MAJOR PATIENTS
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Background: The new parameters of cardiac function, derived from two-dimension sacleck-track echocardiography could be useful for an early diagnosis of cardiac involvement in transfusion dependent β-TM patients.
Aims: In this cross sectional study, our goal was to detect early myocardial disease in transfusion dependent β-TM patients using Echocardiography (Speckle Tracking) to assess its specificity and sensitivity in comparison with cardiac MRI T2*.
Methods: This cross sectional study included 30 transfusion dependant β-thalassemia patients aged between 11-20 years recruited from the Pediatric Hematology Unit, Children Hospital, Ain Shams University. All included patients were subjected to detailed medical history(including transfusion, chelation, hepatitis, C virus history with calculation of mean serum ferritin in last 2years) Radiological investigation included Echocardiography (Tissue Doppler and Speckle Tracking),MRI T2* were done.Cardiac affection by speckled was defined as decreased longitudinal strain less than 11 percentage or affection of any segment less than 11 percentage.
Results: Cardiac affection by speckled echocardiography was found in 10 patients(33.3%), 8 of them (80%) had normal ejection fraction and normal shortening fraction, while 9 had iron overload by Cardiac MRI T2*. Patients with mean serum ferritin >2500 ng/mL in the last 2 years prior evaluation showed a significantly lower longitudinal strain (GLPSLAX) (P=0.043) which was further proved by a significantly negative correlation with the mean serum ferritin (P=0.002). No significant differences were found between both spelenumerized and non spelenumerized patients as regard speckle tracking echocardiographic measures. The ROC curve analysis revealed that GLPS A4C a cutoff value of ≤21% was able to detect B-thalassemia patients having myocardial disease by cardiac MRI T2* with a sensitivity of 87.50% and specificity of 63.64%. Patients with cardiac iron overload by MRI T2* had significantly lower GLPSLAX &GLPSA4C and higher Ao Diam than those without cardiac iron overload (P=0.016, P=0.008, P=0.047 respectively). No significant difference between beta thalassemia patients with cardiac affection and those without cardiac affection as regard the duration of the disease, type and compliance of chelation therapy.
Summary/Conclusions: Although, Magnetic Resonance Imaging T2* technique is the new reference standard for cardiac iron overload, its routine use is limited by its high costs, poor availability. We demonstrated in this study an abnormal global longitudinal strain despite preserved LV systolic functions among BTM patients; thus speckle tracking echo techniques might be considered as an alternative effective method to detect early myocardial disease before evident systolic dysfunction.

E1581
EFFICACY, SAFETY AND GENETIC BASIS OF VARIABILITY OF RESPONSE TO HYDROXYUREA THERAPY IN BETA THALASSEMIA: A SYSTEMATIC REVIEW
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Background: Pharmacological agents such as hydroxyurea promote fetal hemoglobin production via a reactivation of γ-chains. In β-thalassemia there is an imbalance in globin chains which could be ameliorated by the newly synthesized α-chains which neutralize the excess α-chains and therefore improves symptoms.
Aims: Systematic review of literature to evaluate the efficacy, safety and the genetic basis of variability of response to hydroxyurea therapy in beta-thalassemia patients.
Methods: Research sources used were: MEDLINE (PubMed), EMBASE (Ovid) and Cochrane from June 1993 till June 2016. Eligible articles were reviewed and data including patients' characteristics, duration of treatment, outcome, toxicity and impact of genetic mutation on response to hydroxyurea therapy was extracted. Major responders were those who became transfusion independent after hydroxyurea treatment, partial responders had significant decline in transfusion requirements, poor responders did not respond to hydroxyurea therapy. Statistical analysis software package 16 was used for data analysis.

Table 1.

<table>
<thead>
<tr>
<th>Type of Beta Thalassemia</th>
<th>Major Response</th>
<th>Partial Response</th>
<th>Poor Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-thalassemia major</td>
<td>305 (51%)</td>
<td>184 (31%)</td>
<td>17 (3%)</td>
</tr>
<tr>
<td>β-thalassemia intermedia</td>
<td>50 (8%)</td>
<td>37 (6%)</td>
<td>10 (2%)</td>
</tr>
</tbody>
</table>

Results: Thirty eligible studies comprising of a total of 1822 patients with beta thalassemia were identified. Of these (n=9, 30%) evaluated the effect of hydroxyurea therapy on beta thalassemia major patients, (n=11, 36%) evaluated beta thalassemia intermedia patients while (n=10, 34%) included both beta thalassemia major and thalassemia intermedia patients. Mean age of patients was 13.5 years. Mean duration of hydroxyurea therapy was 3.4 years. The mean dose of hydroxyurea was 10mg/kg per day (1.5mg/kg/kg). Table I showing number and percentage of patients having major, partial and poor response to hydroxyurea therapy. Only (n=12, 36%) studies evaluated the role of underlying genetic mutation on hydroxyurea response, out of these (n=6, 50%) studies found no significant correlation while (n=6, 50%) showed a positive correlation between common genetic mutations and hydroxyurea response. Hydroxyurea was found to be well tolerated, only (n=09, 01%) had transient myelosuppression.
Summary/Conclusions: Hydroxyurea is an effective and well-tolerated agent in the management of β-thalassemia (both intermedia and major). It reduces blood transfusion requirements either partially or completely in majority of patients. No significant correlation between response to therapy and underlying genetic mutation was found. More studies are required to fully establish the association of genetic mutation to drug response.

E1582
EVALUATION OF CONTINUOUS BLOOD GLUCOSE MONITORING METHOD FOR DETECTION OF ALTERATIONS IN GLUCOSE HOMEOSTASIS IN BETA-THALASSEMIA PATIENTS
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Background: Glucose metabolism disturbances, among other endocrinopathies, are a common feature of β-thalassemia (both intermedia and major). Pancreatic iron overload and diabetes mellitus (DM) are common in β-TM patients. However, the relationship between iron stores and glucose disturbances is not well defined. Continuous glucose monitoring system (CGMS) enables more diagnostic accuracy and a better achievement of an optimal glycemic control. Aims: To assess the pattern of glucose homeostasis in patients with β-TM and detect early impairment in glucose metabolism and prediabetic state in β-thalassemia patients comparing oral glucose tolerance test (OGTT) and CGM system.
Methods: This cross sectional study was conducted on 200 patients β-TM patients. Patients were studied focusing on transfusion history, transfusion index, iron chelation therapy and compliance to chelation. Complete blood picture, markers of hemolysis, serum ferritin and random blood glucose (RBG) were measured. Patients with RBG ≥140mg/dL were subjected to OGTT, insertion of CGMS for 3 days, measurement of fasting C peptide, and serum insulin with calculation of HOMA-IR and assessment of HbA1c.
Results: Screening with RBG revealed that 20 patients (10%) had RBG ≥140mg/dL. Using OGTT, 7 (3.5%) patients were in the diabetic range, 7 (3.5%) had normal OGTT while 6 (3%) had impaired glucose tolerance. The CGMS showed that 7 (3.5%) patients had IGT (6.5%) and 13 patients had diabetes
mellitus. The percentage of diabetic patients diagnosed by CGMS was significantly higher than that with OGTT (p=0.012). According to CGMS readings, 10 of the 13 patients with diabetes had abnormal HbA1c readings. According to T2*MRI readings, 10 of the 13 patients with diabetes had abnormal HbA1c readings. Serum ferritin was significantly higher among patients with RBB≥140mg/dL (p=0.001). It was noted that 60% of patients with anemia were noncompliant and 75% of patients on desferrioxamine therapy had RBB≥140mg/dL. There was a significant positive correlation between HbA1C% and FBG among the studied thalassemia patients with elevated RBB≥140mg/dL, while HbA1C% was negatively correlated with fasting C-peptide. Serum ferritin was positively correlated with RBB. As regards CGMS data, HbA1C was positively correlated to maximum blood glucose, average blood glucose, SD blood glucose and area under the curve≥140mg/dL. The only significant independent factor for elevated RBB ≥140mg/dL was serum ferritin.

Summary/Conclusions: The use of CGMS in the diagnosis of early glycemic abnormalities (prediabetes) among patients with β-TM appears to be promising and superior to other known diagnostic modalities namely OGTT and HbA1c.

Table 1.

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E1585

ASSOCIATION OF SP1 POLYMORPHISM IN THE COLLAGEN TYPE 1 ALPHA 1 (COL1A1) GENE WITH OSTEOPOROSIS IN CHILDREN WITH BETA-TALASSEMIA

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Background: Osteoporosis is a progressive bone disease that is characterised by a decrease in bone mass and density that leads to an increased risk of fracture. Early detection of mutation at the Sp1-binding site on the COL1A1 gene is mandatory in order to initiate preventive therapy before the occurrence of fractures in children with thalassemia.

Aims: To study the relationship between SP1 polymorphism in the collagen type 1 alpha 1 gene and the development of osteoporosis in patients with Beta thalassemia.

Methods: A prospective case control study was carried out in the Outpatient Clinic of Hematology Unit of Pediatric Department and Clinical Pathology Department at Zagazig University Hospitals on forty thalassemic patients (21 females &19 males) aged 6-18 years during their regular follow-up visits (22 patients with thalassemia major and 18 with thalassemia intermedia) and forty age- and sex-matched healthy children as a control group. All patients and control were subjected to full medical history, thorough clinical examination and laboratory investigations in the form of complete blood count, Hb electrophoresis, Calcium level Serum, alkaline phosphatase, Bone Density by DXA, Serum osteocalcine level and COL1A1 gene polymorphism by using polymerase chain reaction-restriction fragment length polymorphism technique (PCR-RFLP).

Results: There was highly significant difference between thalassemia patients and control group as regards serum levels of calcium, osteocalcin and alkaline phosphatase and DEXA results but no significant difference between thalassemia major and thalassemia intermedia patients. As regard COL1A1 genotype there was high percentage of heterozygous Ss (G/T) and homozygous ss (T/T) genotype in beta thalassemia major 55.63%, 13.67% than thalassemia intermedia 50.6%, 0%, respectively. There was significant relation between COL1A1 genotypes and Calcium level (p=0.02). But there was no significant relation between COL1A1 genotypes and osteocalcin, alkaline phosphatase levels and DEXA among studied groups.

Summary/Conclusions: SP1 polymorphism in collagen gene could be of clinical value in identifying the thalassemic patients at risk of developing osteoporosis.

E1586

UNUSUAL MOLECULAR MECHANISMS IN THE ORIGIN OF ALPHA-TALASSEMIA

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E1587

Abstract withdrawn.

E1588

VALUE OF HBA2 IN THE DIAGNOSIS OF BETA-TALASSEMIA MINOR “ATTENTION TO THE GRAY ZONE”

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Background: The homozygosity for the alternative splicing mutation HBB: IVSI-6 (C>T) is the most frequent genotype of beta thalassemia intermediate in our population and was even termed “beta thalassemia intermediate type Portuguese” (Tamagnini et al, 1983). The IVSI-6 (C>T) carriers (heterozygous) are characterized by mild hypochromia and microcytosis, with a moderately increased in HbA2, that may be even less than 3.5%. The correct identification of these carriers is important, especially when facing a couple who intends to have children.

Aims: To evaluate the percentage of individuals with hypochromia and microcytosis and Hb A2 between 3.2% and 3.4%, who are beta thalassemia carriers, alerting for the need to adapt the cut-offs of HbA2 values to the genetic background of different populations.

Methods: Parameterized search of all the consecutive individuals evaluated in our laboratory from January 2007 to January 2016. The inclusion criteria were the simultaneous presence of hypochromia and microcytosis (adjusted to the age) and HbA2 values between 3.2% and 3.4% inclusive. The exclusion criteria were the presence and/or clinical information of sideropenia or sideropenia anemia, hemoglobin variants or alpha thalassemia. Sequencing of the entire HBB gene was performed by Sanger Sequencing.

Results: Respecting the inclusion and exclusion criteria we have identified 43 individuals with hypochromic and microcytic anemia, HbA2 ≥3.2% and ≤3.4%, in which the HBB gene mutations were screened. Among the 43 subjects, nineteen presented HbA2 ≥3.2% (19/43), eleven HbA2 ≥3.3% (11/43) and thirteen had HbA2 ≥3.4% (13/43). The IVSI-6 (C>T) mutation was identified in 2 subjects with HbA2 ≥3.2% (10%), 5 with HbA2 ≥3.3% (45%) and 7 with HbA2 ≥3.4% (54%). No other HBB gene mutations were detected. The remaining individuals were classified as probable alpha thalassemia and suggested continuation of the study, if warranted.

Summary/Conclusions: We have identified 14/43 (32%) individuals as beta thalassemia carriers who, for the conventional cut-off of HbA2 ≥3.5%, would not have been diagnosed. Based on this data, we propose that individuals with hypochromia and microcytosis, with normal or slightly elevated RDW, without sideropenia, with HbA2 between 3.2-3.4%, should be screened for mutations in the HBB gene, in order to rule out beta thalassemia carriers due to Beta+ mutations. As HBB IVSI-6 (C>T) mutation is one of the most frequent beta thalassemia mutations in Portugal, and in Mediterranean basin, it is necessary to keep in mind the classic rule of HbA2 ≥3.5% for the diagnosis of beta thalassemia minor may underdiagnose this pathology and lead to an incorrect genetic counseling.

E1589

DIAGNOSIS OF HEMOGLOBINOPATHIES BY CAPILLARY ZONE ELECTROPHORESIS: EXPERIENCE WITH 925 CASES

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Background: Hemoglobin capillary zone electrophoresis is a relatively newer technique as compared to HPLC for detection of abnormal hemoglobins. We share our first hand experience of using Capillary 2 Flex piercing instrument for diagnosis of hemoglobinopathies as a primary diagnostic modality.

Aims: The main aim was to evaluate a new technology for diagnosis of hemoglobinopathies.

Methods: The capillary 2 Flex piercing instrument with Phoresis software for hemoglobin electrophoresis at alkaline pH was evaluated at our centre over a period of 1 year. A total of 925 sample runs were included in the analysis. The equipment was assessed on the following parameters: ease of operation, pre-analytical factors, identification, quantification and precision of hemoglobin variants including the rare variants. Further, we evaluated if capillary zone electrophoresis can be useful as a single method for diagnosis of hemoglobinopathies.

Results: The automation provided by capillary zone electrophoresis eased the problem of errors during sample preparation. The option for low sample volume mode is a great help in samples from children. The instrument could readily identify all common hemoglobinopathies and the diagnosis was straightforward in 829 (89.7%) cases. In the rest 96 (10.3%) cases, the sample was required to be rerun because it lacked Hb A or Hb A2. This posed inconvenience because the electrophoretic zones get displaced and have to be derived after mixing it with normal sample. The machine is not specifically standardized for cord blood samples hence we are not performing tests on neonatal cord blood sample. The instrument could separately identify Hb E from Hb H (1.2% and 0.9% ) on HPLC. Hemoglobins falling into the same zone and requires other modalities to confirm. Two cases where Hb H was strongly suspected clinically and HB H inclusion test was positive showed small peaks over HPLC, however, we found mild high Hb A2 both in heterozygous and homozygous Hb E cases ( heterozygous Hb E, n-28 mean Hb A2- 3.9% and homozygous Hb E, n- 7 and mean Hb A2- 4.2%) leaving the doubt whether some adducts are still left. Identification of small peaks of Hb H could be difficult and requires other modalities to confirm. Two cases where Hb H was strongly suspected clinically and HB H inclusion test was positive showed small peaks of HB H (1.2% and 0.9% ) on HPLC. Hemoglobins falling into the same zone (eg Hb D- Punjab and Hb Q India) needed identification with second modality. Whenever encountered with problem of identifying certain abnormal peak, we resorted to HPLC for confirmation. Spectrum of hemoglobin variants encountered (n-298 cases, rest 627 showed normal results) in the study is listed in table below.

Table 1.

<table>
<thead>
<tr>
<th>Type of Hemoglobin</th>
<th>No. of cases (n = 298)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Beta thalassemia</td>
<td>35</td>
</tr>
<tr>
<td>2. Hemoglobin E</td>
<td>6</td>
</tr>
<tr>
<td>3. Hemoglobin H</td>
<td>207</td>
</tr>
<tr>
<td>4. Hemoglobin D</td>
<td>58</td>
</tr>
<tr>
<td>5. Hemoglobin Q</td>
<td>34</td>
</tr>
<tr>
<td>6. Hemoglobin C</td>
<td>20</td>
</tr>
<tr>
<td>7. Hemoglobin A</td>
<td>20</td>
</tr>
<tr>
<td>8. Hemoglobin S</td>
<td>12</td>
</tr>
<tr>
<td>9. Hemoglobin F</td>
<td>10</td>
</tr>
<tr>
<td>10. Compound heterozygotes (eg Hb H, Hb A2, Hb E)</td>
<td>16</td>
</tr>
</tbody>
</table>

Summary/Conclusions: Capillary zone electrophoresis is an alternative method for hemoglobinopathy. However, since the diagnosis of Hemoglobin variants mandates confirmation by a second method, HPLC cannot be replaced completely. Based upon the availability, workload and cost effectiveness, any of these two methods can be used as primary modality.
Thrombosis and vascular biology

E1590

RELEVANT ROLE OF VON WILLEBRAND FACTOR-ADAMTS13 AXIS IN HEPATIC ISCHEMIA-REPERFUSION INJURY

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HEPATIC ISCHEMIA-REPERFUSION INJURY: RELEVANT ROLE OF VON WILLEBRAND FACTOR-ADAMTS13 AXIS IN ARTERIAL THROMBOSIS

M. Scully1

Background: Hepatic ischemia-reperfusion (I/R) injury is a liver damage occurring during liver surgeries such as hepatic resection or transplantation, and denotes the major basis for graft dysfunction after transplantation. Although detailed mechanisms of hepatic I/R injury remain to be clarified, an excessive inflammatory response is thought to play a role in this regard.

Aims: Since recent studies suggest that von Willebrand factor (vWF) plays a pivotal role in a cross-talk between inflammation and thrombosis, we assumed that vWF may be involved in the pathophysiology of hepatic I/R injury. To test this hypothesis, we have used a mouse experimental model of hepatic I/R injury.

Methods: Mice were anesthetized with sodium pentobarbital and a midline laparotomy was then performed on a heating pad. Blood supply for the left lateral and median lobes of liver (approximately 70% of the liver mass) was interrupted by cross-clamping the hepatic artery and portal vein with a microvascular atrumatic clip for 90 min. Then a clip was taken off to provoke the reperfusion of hepatic blood flow, which was monitored on the surface of left lateral lobe by Laser Doppler flowmetry (LDF, ALF21, Advance Co, Tokyo, Japan). The hepatic blood flow was measured again 24 h after reperfusion and mice were then sacrificed. Histological and histochemical analysis of liver tissue sections were performed.

Results: 193 TIA (31.6%) and 38 haemorrhagic stroke (6.2%). 161 (26.4%) had abnormalities in haematocrit or platelet count: 116 (19%) had a raised haematocrit, 19 (3.1%) thrombocytosis, and 26 (4.2%) thrombocytopenia. Of these, 7 patients demonstrated abnormalities of both cell lines. Of these initial 161 abnormal results, 119 (73.9%) were repeated but 42 (26.1%) were not. JAK II testing was deemed warranted in 17 (2.8%): a persistently raised or progressively raised haematocrit or platelet count respectively, with normal liver and renal function and no other explicable cause. JAK II mutational analysis was performed in 3 patients (0.5%). One was proven positive for the V617F mutation, hence diagnosed with polycythemia vera. Of the 2 negative JAK II results, one patient was subsequently diagnosed with chronic myeloid leukaemia.

Summary/Conclusions: In stroke patients <60 years, one quarter had abnormalities in haematocrit or platelets. Myeloproliferative disease or TTP was present in 3 patients of 5 specifically investigated in the cohort. From a haematological perspective, at least 21 further patients merited further investigation.

We retrospectively reviewed full blood counts, specifically haematocrit and/or platelet count, and whether these were documented and further investigated. Although primary haematological disorders are rare as a cause of thrombocytopenia, defined as a persistently thrombocytopenic patients, defined as a persistently raised thrombocytopenia, ADAMTS13 testing was not warranted in 17 of these (subsequent resolution of platelet count n=7, HIV = n=2, liver derangement n=7, known ITP with no MAHA n=1). ADAMTS13 testing was indicated in 9 of these patients (34.6% of thrombocytopenic patients), defined as a persistent thrombocytopenia with no clear cause, normal liver and renal function and negative HIV status. Seven of these patients did not have ADAMTS13 considered, according to the clinical documentation, nor sent. Of the 2 tested for ADAMTS13, one result was normal, helping to resolve the clinical diagnosis of ITP. In the other patient, ADAMTS13 was <5%, confirming TTP and facilitating prompt treatment.

E1591

THE IMPORTANCE OF THE FULL BLOOD COUNT, JAK II AND ADAMTS13 TESTING IN STROKE EVALUATION: A REVIEW OF 619 CONSECUTIVE YOUNG STROKE AND TIA PATIENTS

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Background: Thromboembolic events in young stroke and TIA patients are rare and further analysis for TTP is recommended. However, this number may be higher since a quarter of those patients with initial discrepancies of haematocrit and/or platelet count did not have repeated testing. Although primary haematological disorders are rare as a cause of stroke, a basic full blood count result should not be ignored in considering the aetiology of arterial thrombosis in a younger cohort.

Aims: To analyze the incidence of PRT, describe the clinical characteristics and management of these patients and identify the risk factors of PRT.

Methods: We performed a retrospective chart review of 230 adult patients diagnosed with hematological malignancies, in whom, experimented nurses tuned PCCs with different techniques: blinding Seldinger from 2010 to 2014 and guided by ultrasonography (US) from 2015 to 2016. PRT diagnosis was confirmed by Doppler US. Statistical analysis was performed using the SPSS version 20.

Results: The median age was 58 years (14-86) and 55.7% of the patients enrolled in the study were male. The most frequent hematological malignancies were: Non-Hodgkin’s lymphoma (NHL=105; 45,7%) myeloid malignancies (acute myeloid leukemia and myelodysplastic syndromes=60; 26,1%), acute lymphoblastic leukemia (ALL=22; 9,6%), multiple myeloma (MM=19; 8,3%) and Hodgkin lymphoma (HL=17; 7,4%). In 188 patients (82%), PCC was tunneled when the active disease was presented. Only 51 patients (22%) received thromboprophylaxis based on low molecular weight heparin (LMWH=27), aspirin (ASA=21) or vitamin K antagonist (VKA=3). PCCs were tunneled guided by US in 127 patients (55,2%), and the main location of tip catheter was in cava-right atrium region (66%). The overall incidence of PRT was 7% (n=16). The main diagnoses related to PRT were ALL (6), NHL (5), myelodysplastic syndromes (MDS=3) and multiple myeloma (MM=2). All discharge rates were increased: 30-day mortality = (15/16=94%). Fourteen patients (88%) were treated by chemotherapy based in L-asparaginase (L-ASA), immunomodulatory drugs or other treatment combined with corticosteroids. The median onset of PRT was 26 days (range: 0-230) and 8 of them (50%) in the first 30 days after insertion. In 11 cases (69%) PCC was removed within 72 hours of PRT and treated with LMWH to a median of 4 months (range: 1-11). During follow-up, no patient had progression of thrombosis, or pulmonary thromboembolism. Finally, in the univariate analysis ALL, HL and L-ASA had significant impact on PRT.
PEDIATRIC VENOUS THROMBOEMBOLISM: INCIDENCE, RISK FACTORS AND GENETIC BACKGROUND BETWEEN POPULATIONS.

BACKGROUND: Venous thromboembolism (VTE) is a multifactorial disease caused by the interaction of acquired required risk factors and complex gene-gene and gene-environment interactions. VTE results from the development of a thrombus, usually in the deep veins of the leg (deep vein thrombosis, DVT) that can subsequently embolise to the lung (pulmonary embolism, PE). Classical inherited risk factors for VTE in European-ancestry populations include protein C and S deficiencies, factor V Leiden and prothrombin gene mutation (FII G20210A). Several other common and low-frequency susceptibility variants, mainly single nucleotide polymorphisms (SNPs) in loci ABO, FII, FV, FGG, GP6, KG51, PROCR, SLCA4A2, STXBP5, TSPAN15 and VWF, have been also found robustly associated with VTE. However, in the Portuguese population, the genetic background for VTE for most of these genetic susceptibility variants remains to be evaluated.

AIMS: To investigate the association of five SNPs in the loci ABO (rs2519093 and rs8176719), FII (rs2036914 and rs2289252) and FGG (rs2068665) with VTE in a sample of Portuguese patients.

METHODS: A retrospective (2012-2015) case-control study with 119 cases of unprovoked VTE and 148 healthy controls of Portuguese origin was conducted, to evaluate allele frequencies of the five risk VTE alleles in the Portuguese population and to assess the association between these alleles and the risk for VTE. FXI (rs2036914 and rs2289252) and FGG (rs2068665) SNPs were genotyped by real-time PCR with TaqMan probes. ABO rs2519093 and rs8176719 SNPs were genotyped by restriction fragment length polymorphism (RFLP). PLINK software was used to determine the allelic frequencies, concordance with the Hardy-Weinberg equilibrium and association between risk alleles and VTE through logistic regression, in the additive model, estimating OR with 95% confidence intervals (95% CI) and p-values. The association between the cumulative number of risk alleles and the risk of VTE was assessed using Pearson χ² using the Simple Interactive Statistical Analysis software (SISA).

RESULTS: The estimated risk allele frequencies in the overall study population sample were: 0.212 for FGG rs2068665 (T), 0.62 and 0.50 for FII rs2036914 (C) and rs2289252 (T), respectively, and 0.295 and 0.417 for ABO rs2519093 (T) and rs8176719 (C), respectively. The genotype distributions were in agreement with the Hardy-Weinberg equilibrium for all SNPs. The risk allele frequency regression under an additive model showed that FGG rs2068665 was associated with VTE (nominal p=0.029; OR=1.57, CI 95% 1.05-2.37) as well as ABO rs8176719 (nominal p=0.0064; OR=1.65, CI 95% 1.15-2.36). Both SNPs remain significantly associated, even adjusting for age and sex (P=0.019 and P=0.005, respectively). ABO rs2519093 did not reach significant associations with VTE in our population sample (P=0.184) as well as FII rs2036914 and rs2289252 SNPs (P=0.76 and P=0.16, respectively). In addition, there was an increased risk of VTE associated with the increment in the total number of risk alleles: 0 vs 1 risk allele: χ²(5)=8.5, p=0.015, OR=2.31; and 0 vs 2 or more risk alleles: χ²(2)=12.2, p=0.0048, OR=3.36.

SUMMARY/CONCLUSIONS: Our data suggest that the alleles FGG rs2068665 T and ABO rs8176719 C may contribute to the VTE susceptibility in the Portuguese population. The absence of significant associations for the remaining loci could be the result of a limited statistical power, consequence of a modest effect size of polymorphisms or lower sample sizes, or because of differences in genetic backgrounds between populations.

E1594

PEDIATRIC VENOUS THROMBOEMbolISM: INCIDENCE, RISK FACTORS AND MANAGEMENT OF HOSPITALIZED PATIENTS IN A TERTIARY CARE TEACHING HOSPITAL

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BACKGROUND: Venous thromboembolism (VTE) is a considered a rare event in childhood. In spite of this, the incidence of VTE is on the rise in hospitalized patients. Medical progress in the treatment of critically ill patients has increased the incidence of venous thromboembolism (VTE) and interventional procedures, especially in children with cardiac defects and malignant disease. Therefore VTE is increasingly recognized as a major secondary complication of advanced tertiary care in infants and children.

AIMS: To study the incidence, demographics, risk factors, diagnostic tests, therapy, and complications of pediatric acute VTE in our tertiary care hospital.

METHODS: A retrospective single-center study of patients<18 years of age who were discharged from January 2014 to December 2016 by using diagnostic codes for acute VTE from our hospital database. We studied demographic characteristics, clinical presentation, diagnostic tests, risk factors, treatment strategies and outcome.

RESULTS: We report an incidence rate of 10.7 cases per 10,000 patient-years (70 acute VTE events / 21,892 discharge cases over a 3-year period). Patients were predominantly male (57%). Mean patient age was 3.5 years, with the greatest proportion of cases in the infant cohort (32.6%), while children above 1 year comprised 37% and neonates (<1 month) formed 8.6% of our sample. Patients were mostly born at full term (71.4%), although 45.7% of the neonatal and infant cases were premature. Catheter-related (CVC-VTE) comprised 55.7% of VTE cases. On the other hand, non-catheter-related (NCR) diagnoses were more frequent in intracranial in 35.3% of cases. Mean time from admission to deep vein thrombosis (DVT) was 29 days and intracardiac in 19.3%. Only 3 cases of NCR-pulmonary embolism (PE) and 2 cases of NCR-upper extremity DVT were reported. Doppler ultrasound was the most common diagnostic test used (75.7%), followed by MRI, CT and CT angiography in equal proportions. Critically ill patients encompassed most of the cases (88%). Mean duration of hospitalization was 89 days (range 2-156) and time from admittance to VTE diagnosis was 25.6 days. A large proportion had congenital heart defects (32.9%) requiring interventional procedures. Half of the patients (51.4%) had surgery around the time of VTE diagnosis. Malignancy was identified in 5 cases (2 of which were CVC-VTE). Transient triggers, such as infection (12 cases) and use of aspiraginase (2 cases) were also reported. Most patients were not tested for thrombophilia (n=44, 62.9%) since they were classified as provoked VTE and from those who were tested 10% were diagnosed with a thrombophilia. A prior transfusion for one patient initiated anticoagulant therapy: 78.6% (n=52) were initially treated with low molecular weight heparin (LMWH) and while most continued treatment with LMWH, 8.6% (n=6) received vitamin K antagonists and 8.6% received direct oral anticoagulants. LMWH dosing was adjusted using anti-Xa assays (AXA) in 85.7% of cases, documenting a median of 5 AXA per patient, out of which 3 were within therapeutic range. Mean duration of treatment was 5.8 months. Recurrence rate was 17%, half of which were in patients with CVC-VTE. On the other hand, bleeding rate was 15.7% most of which were mild (10%) or provoked bleeds (4.3%). Mortality was 10%, although cause of death was not directly related to VTE in any of the cases.

SUMMARY/CONCLUSIONS: Pediatric VTE is a substantial complication arising from tertiary care hospitalization where critically ill infants are at greater risk. Potential risk factors of VTE include use of CVCs, patients with complex congenital heart defects, surgical procedures, infection and malignancy. Further studies on VTE prophylaxis and identification of VTE predictors in a critical care setting are required.

E1595

CELL-BASED EVALUATION OF CHANGES IN COAGULATION ACTIVITY INDUCED BY ANTI-NEDOL Drugs for the Treatment of Acute Myeloid Leukemia

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Background: Idarubicin (IDR), a derivative of (Arac), and lomustine (Amo) are effective for treatment of acute myeloid leukemia (AML). In leukemic cells, the incidence of venous thromboembolism or disseminated intravascular coagulation is associated with induction chemotherapy.

Aims: How some drugs for the treatment of AML affect the procoagulant activity is unclear. Thereby, in this study, we investigated the procoagulant effects of IDR in comparison with Arac and Amo.

Methods: Procoagulant effects of IDR, Arac, and Amo were investigated in a vascular endothelial cell Line926 and AML cell lines HL60 (AML M2), NB4 (AML M3, APL), and U937 (AML M5), focusing on tissue factor (TF), phosphatidylserine (PS), and thrombomodulin (TM). Normal human plasma-based recalcification time assay, flow cytometry analyses, and RT-PCR are applied for the evaluation.

Results: IDR induced procoagulant activity on the surface of vascular endothelial and AML cell lines. Expression of TF antigen, TM antigen, and PS were induced by IDR on the surface of each cell line, whereas expression of TF and TM mRNAs were unchanged. Increased TF and PS expression may overcome increased TM expression and the overall effect may be procoagulant. Conversely, Amo decreased TF expression and procoagulant activity, and increased TM expression on NB4 cells. In NB4 cells, we observed downregulation of TF mRNA and upregulation of TM mRNA by Amo. But Amo did not sufficiently inhibit anticoagulant activity on NB4 cells when used simultaneously with IDR.

Summary/Conclusions: These data suggest IDR may induce procoagulant activity in vessels by apoptosis through PS expression and/or TF expression on vascular endothelial and AML cell lines. Amo may suppress procoagulant changes by downregulation of TF expression and induction of TM expression. Our methods could be useful to investigate changes in procoagulant activity induced by antineoplastic drugs.
E1596 DESCRIPTION OF THROMBOTIC EVENTS AND/OR PREGNANCY LOSS IN A COHORT OF HOMOZYGOUS CARRIERS FOR THE C46T POLYMORPHISM IN THE F12 GENE
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Background: The intrinsic pathway of coagulation is initiated by a serine protease named factor XII (FXII) in a reaction involving the contact system and triggers fibrin formation through activation of factor XI. In vitro, FXII triggers activation of the classic complement pathway and initiates the fibrinolytic system via plasma kallikrein mediated urokinase activation, whereas in vivo its role is not clear. Factor XII (FXII) is a nucleotide triphosphate diphosphohydrolase in the 5’-untranslated region of the F12 gene (F12 C46T) is associated with lower levels of FXII. Its frequency varies widely across populations and ethnic groups, ranging from 0.18 in the Spanish population to 0.67 among Japanese. Homozygosity for the C46T polymorphism of the F12 gene has proved to be an independent risk factor for thrombosis and unexplained recurrent spontaneous abortion. However, the precise role of this polymorphism as a thrombotic risk factor is controversial, and the evidence for an association between F12 C46T, venous thromboembolism (VTE) and myocardial infarction is weak.

Aims: To describe the occurrence of thrombotic events and/or pregnancy losses in a cohort of homozygous individuals for F12 C46T.

Methods: We retrospectively analyzed all the homozygous F12 C46T cases diagnosed in our laboratory from January 2015 to January 2017. Allelic discrimination using real time PCR confirmed the presence of homozygous F12 C46T mutation. The following variables were collected: age, gender, race, cardiovascular risk factors (CVRF) (hypertension, diabetes mellitus, dyslipidemia, smoking and overweight), history of cancer, VTE (type, recurrence, treatment, complications), hospitalization, thrombosis, number of pregnancy losses and other inherited/acquired thrombophilia.

Results: 122 cases were evaluated: 45 (36.88%) male and 77 (63.12%) female. Mean age: 46.2 years (1-86). Race: 65.57% caucasian, 13.1% american, 2.4% black, 1.6% asian, 4.1% other. Decreased factor XII plasma levels were found in 81.42% of them, with mean factor XII levels 53.73% (27.5-107.5). Overall, 34.48% of the subjects had at least one thrombotic event. Type of thrombosis: 64.4% VTE and 35.6% arterial thrombosis. One (26.7%) or more than one (46.7%) additional thrombotic risk factors were found in patients with any thrombotic event. Presence of one or more CVRF was found in 66.7%. Familiar history of thrombosis was found in 16%, whereas 13% had a recent or active malignant neoplasm. Among women, 28.57% and 12.98% had one and more than one pregnancy loss respectively. Additional thrombotic risk factors were found in 66% of women with recurrent losses. One (43%) or more than one (57%) additional thrombotic risk factors were found in women with any pregnancy loss. Presence of one or more CVRF were found in 30% of them. Familiar history of thrombosis was found in 34.7%, whereas none of them had a recent or active malignant neoplasm.

Summary: Most patients with a thrombotic episode had one or more additional risk factors. Nevertheless, up to 26.7% presented no other risk factor than homozygous F12 C46T, suggesting a relevant role in the pathogenesis of thrombosis. According to our results, the risk of abortion could be increased by the presence of homozygosity for F12 C46T, since it was the only thrombotic risk factor in women with recurrent pregnancy losses. Further studies are needed to clarify the real contribution of F12 C46T to thrombosis and pregnancy losses on prospectively selected patients.

E1597 ANALYSIS OF CHARACTERISTICS OF HOSPITAL ASSOCIATED THROMBOSSES
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Background: Hospital associated thrombosis (HAT) is now commonly monitored but expected targets of HATs remains poorly reported.

Aims: We analysed HATs in our hospital group over a 40 month period to identify those patients with hospital-associated thrombosis (HAT), defined as patients having had a hospital inpatient episode, including day case surgery and admissions of 4-24 hours, in the 90 days prior to their VTE episode. Root cause analysis was undertaken on these cases, recording information of the index episode, investigating additional risk factors performed and the corresponding prescribing and administration of thromboprophylaxis (TP) consisting of LMWH or GCS.

Results: A total of 2222 VTEs were identified (1051 PE’s and 1178 DVTs) of which 581 (26%) HATs were identified (312 PE’s, 269 DVTs). This represents an excess of PE’s over the expected rate based on total VTE distribution between PE and DVT (p=0.0002 Fishers exact test). The majority of patients had a medical (non-surgical) index admission with 58.5% admitted as acute medical admissions and 41.5% surgical admission (trauma and orthopaedics 18.4%, general, vascular and GI surgery 12.2%, urology 4% and Gastroenterology and gynaecology 2%). Not all surgeries had thrombo prophylaxis. In 526 HAT cases, root cause analysis (RCA) revealed that 101 (19.2%) were deemed preventable and 367 (69.8%) were not thought to be preventable. The remaining 57 cases had the index admission outside of our trusts, largely having undergone pre-hospital procedures which were not recorded.

Of 394 HAT cases with sufficient data, 80 (20.3%) had a preventable cause, 27 receiving insufficient TP, 9 receiving delayed TP, 26 having no TP given though indicated and 18 not having a VTE risk assessment. Some cases of insufficient TP were deemed due to underrating standard patient >90kg. Off those HAT cases deemed preventable, 37 patients had contraindications to TP, 166 had TP failure i.e. full TP given and in 102 TP was not indicated. 9 patients were on full anticoagulations at time of index admission.

Summary/Conclusions: HAT rates remain stable and the majority are though clinical events by current techniques. Key errors reported are failure to perform a timely VTE risk assessment and action with appropriate thrombo prophylaxis. Full integration of electronic patient records with electronic prescribing modules may reduce further these errors.

E1598 THROMBOSIS DURING INFANCY AND NEWBORN PERIOD: AN UNRESOLVED ISSUE
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Background: Reported incidence of thrombosis is higher among newborn infants that can be explained by age related deficiency of anticoagulants, over production of procoagulants and deficiency of fibrinolytic in addition to exposure to multiple risk factors and wide use of catheters which may eventually lead to the transient prothrombotic phenotype in this age group.

Aims: Our aim is to evaluate clinical and laboratory data, risk factors, outcomes of infants (<1-12 months) and newborns (<1 month) with thrombosis in our center.

Methods: Our database revealed 752 children having various types of thrombosis between January 2003 to December 2015 and 77 out of 752 were diagnosed as thrombosis under one year of age. We retrospectively evaluate their clinical, laboratory results and entries in their electronic medical files.

Thrombotic risk factors included inherited and acquired hypercoagulable states, catheter, infection, trauma, surgical operations were also recorded.

Results: There were 51 male and 26 female with a median age of 4 months (0-12 months) in this group. Among 77 thrombotic events 22 (28%) were observed during the infancy period (≤1 month) with a male predominance (n=15, 68%) and from those 22 events 2 were arterial thrombosis (purpura fulminans(1), cerebral(1)) whereas 4 intracardiac, 5 sinusovenous and 11 venous thrombosis (deep veins(4), renal veins(3), portal veins(3), cerebral vein(1)) were noted. In 2(9%) cases thrombosis was diagnosed on the first day of life and 11 out of 22 patient had underlying risk conditions such as prematurity(3), perinatal hypoxia(2), necrotizing enterocolitis(1), congenital cardiac disorders(3), congenital nephrotic syndrome(1) and adrenal insufficiency(1). Moreover 6 out of these 22 thrombotic event catheter insertion was the associated risk factor and 4/22 had infection.

Factor V Leiden mutation was found to be homozgyous in 1/18 and normal in 17/18. Heterozygous prothrombin 20210A mutation were detected in 1 out of 18 and homozzygous MTHFR C677T mutation was found in 3/13 patient. Half of them (12/54) were initially treated with LMWH and TPA were used as a thrombotic agent in 5 case with confirmation of thrombotic diagnosis. During the follow up period 1 patient had an amputation, 5 patient deceased; one because of sepsis and the rest 4 had primary dissease and thrombosis. The site of location in 55 thrombotic events during the infancy period involved deep venous thrombosis (22), cerebral sinusovenous thrombosis (10),cardiac(8), portal(3), renal(1) veins and cerebral arterial(7), femoral arterial(3),abdominal aortic thrombosis(1).In this group 42(76%) out of 55 had an underlying disorder and most common associated risk factor for this age group was inserted catheter related thrombosis, infection and surgical operations. Initial treatment choice was LMWH in 25(45%) and during the follow up period 10 had transition to warfarin. In 21 resolved, 10 had paries thrombosis, 4 deceased and 10 loss to follow up.

Summary/Conclusions: During the first month of life thrombotic complications is 40 times higher than at any other pediatric age. As previously reported venous thrombosis which mainly affect the limbs, the right atrium and renal veins are more frequently seen than arterial thrombosis in newborn infants with a male predominance is compatible with our findings. In the absence of randomized clinical trials the choice of anticoagulation and the duration of treatment for this age group is still controversy beside the complex mechanism and a high mortality& morbidity rate. Although clinical and laboratory data of neonates were compatible with infants, treatment choices differ between these two groups and it seems that thrombolytic treatment was tend to be used more commonly in the neonates without any complication.

E1599 THE QUALITY COMPOSITION OF SOLUBLE FIBRIN MONOMER COMPLEX FRACTION FOR ACUTE AND POST ACUTE ISCHEMIC STROKE PATIENTS
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1Neuropathologists, Haematology

22nd Congress of the European Hematology Association
and the reading was done independently by two different technicians or biologists.

Methods: SFMC fraction was obtained from acute ischemic stroke patients and one year post acute phase of stroke in the absolutely the same patients.

Aims: We explored the quality difference between the SFMC fraction obtained from acute ischemic stroke patients and one year post acute phase of stroke in the absolutely the same patients.

Methods: SFMC fraction was obtained from each tested groups: 35 healthy donors as well as 66 patients with atherothrombotic ischemic stroke (AIS) and 56 patients with cardioembolic ischemic stroke (CIS) during the acute phase of disease; 56 patients with AIS and 56 patients with CIS one year past acute phase. SFMC were collected from blood plasma of each tested subtypes of ischemic stroke by incubation with 0.78% o-phenanthroline per 5 min. For Size-exclusion chromatography, SFMC in volume ml was applied on Healthcare Life Sciences “HLoad 16/60 Superdex 200 pg” column.

Results: Results suggest presence of proteins with Mr from 45 up to 330 kDa in SFMC fraction. The content of SFMC was similar for all stroke fractions with some exception. The difference between results of separation of stroke fractions and fractions obtained from healthy donors was obvious. Mostly the proteins content of the SFMC fraction is similar for stroke and healthy fractions. But amount of the proteins as mean peaks high is different (Figure 1). In fact, the first three peaks which correspond to the 330, 280 and 250 kDa of chromatogram of SFMC are common for all tested fractions and were verified only in their height. Accordingly, the most widely represented variations peaks for AIS, even a year after stroke soluble fibrin monomer complex content was higher comparing to the healthy donors index. Healthy donors also had some of these complexes, but in trace amounts. For acute CIS situation was similar as for AIS, but past one year it got closer to healthy donors.

Figure 1. Summary/Conclusions: It was shown that development of ischemic stroke accompanied by the formation of SFMC in the bloodstream that could take part in disease complication.

E1600 EVALUATION OF A RAPID NANOPARTICLE-BASED LATERAL FLOW IMMUNOASSAY (STIC EXPERT HIT) FOR THE DIAGNOSIS OF HEPARIN-INDUCED THROMBOCYTOPENIA IN A CARDIOTHORACIC HOSPITAL G. Stouff1,2, M. Katalygoragi2,1, S. Georgantis1, S. Kostelidou1, T. Kanellopoulou1, G. Soufla1,*, N. Smith1

Background: Soluble fibrin monomer complexes (SFMC) are the early marker of thrombophilia that represent the complexes of monomeric fibrin with fibrinogen or their products of degradation (FDP). SFMC levels are not directly affected by therapy with thrombolytic agents. Detection of SFMC formed due to the activation of blood clotting by thrombin reveals a pathological process in the early, preclinical stages.

Aims: We explored the quality difference between the SFMC fraction obtained from acute ischemic stroke patients and one year post acute phase of stroke in the absolutely the same patients.

Methods: SFMC fraction was obtained from each tested groups: 35 healthy donors as well as 66 patients with atherothrombotic ischemic stroke (AIS) and 56 patients with cardioembolic ischemic stroke (CIS) during the acute phase of disease; 56 patients with AIS and 56 patients with CIS one year past acute phase. SFMC were collected from blood plasma of each tested subtypes of ischemic stroke by incubation with 0.78% o-phenanthroline per 5 min. For Size-exclusion chromatography, SFMC in volume ml was applied on Healthcare Life Sciences “HLoad 16/60 Superdex 200 pg” column.

Results: Results suggest presence of proteins with Mr from 45 up to 330 kDa in SFMC fraction. The content of SFMC was similar for all stroke fractions with some exception. The difference between results of separation of stroke fractions and fractions obtained from healthy donors was obvious. Mostly the proteins content of the SFMC fraction is similar for stroke and healthy fractions. But amount of the proteins as mean peaks high is different (Figure 1). In fact, the first three peaks which correspond to the 330, 280 and 250 kDa of chromatogram of SFMC are common for all tested fractions and were verified only in their height. Accordingly, the most widely represented variations peaks for AIS, even a year after stroke soluble fibrin monomer complex content was higher comparing to the healthy donors index. Healthy donors also had some of these complexes, but in trace amounts. For acute CIS situation was similar as for AIS, but past one year it got closer to healthy donors.

Figure 1. Summary/Conclusions: It was shown that development of ischemic stroke accompanied by the formation of SFMC in the bloodstream that could take part in disease complication.

E1602 THE IMPORTANCE OF PLATELET MEMBRANE FLUIDITY AND OXIDATIVE STRESS IN THROMBOTIC COMPLICATIONS ACQUIRED BY CHRONIC MYELOPROLIFERATIVE NEOPLASMS PATIENTS V.M. Popov1,*, M. Andreeuc2, M. Omer2, A. Trifa2, F. Miha1, C. Dragan1, G. Patrinol1, M.G. Moisescu 2, T. Savopol 2, E. Kovacs 2, H. Bumbea 3, A. Trifa1, F. Mihai1, C. Dragan1, M. Andreescu1, M. Omer1, A. Trifa1, F. Mihai1, C. Dragan1, M. Omer1, A. Trifa1, F. Mihai1, C. Dragan1, M. Omer1, A. Trifa1, F. Mihai1, C. Dragan1, M. Omer1, A. Trifa1, F. Mihai1, C. Dragan1

Background: Heparin Induced Thrombocytopenia (HIT) is a severe complication of heparin anticoagulation treatment that could be life threatening. HIT diagnosis is therefore of crucial importance in clinical practice especially for the cardiothoracic patients that are often exposed to heparin before surgery. The diagnosis of HIT syndrome with the ‘4Ts’ scoring system.

Methods: Stic Expert HIT, a rapid-nanoparticle based lateral flow immunosay was performed on plasma from 35 patients from July 2016 until January 2017 and the reading was done independently by two different technicians or biologists.

Aims: We assessed a rapid nanoparticle-based lateral flow immunosay (Stic Expert HIT) for assessing the presence of IgG antibodies to PF4/Heparin in patients plasma or serum in cases of emergency diagnosis of HIT needed for patients requiring urgent cardiothoracic surgery over a six-month period.

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Aims: We assessed a rapid nanoparticle-based lateral flow immunosay (Stic Expert HIT) for assessing the presence of IgG antibodies to PF4/Heparin in patients plasma or serum in cases of emergency diagnosis of HIT needed for patients requiring urgent cardiothoracic surgery over a six-month period.
different kind of treatment. Production of ROS was examined using flavorescent method with DCFDA and Fluorolog spectrophotometer. Platelet receptor expression was evaluated by flowcytometry method studying adhesion marker (CD 42 and CD 42b) and aggregation marker (CD61, CD41).

**Results:** Patients with MPN and JAK2 mutation present a high level of fluorescence anisotropy compared with the JAK-negative group. Median value for JAK2 positive group 147.2% CI for median value (157.7-150.6) vs JAK2 negative MPN group 130.8 (124.6-138.3) p<0.001. There are no differences between CML and MPN group. Our results confirm that fluorescence anisotropy is influenced by medication taken. MPN patients who have taken Hydroxyurea alone had a high level of fluorescence anisotropy, median value and 95% CI for median value 151 (137.1-158.6) vs 136 (126-137.5) p=0.03. A patient who have treatment with tyrosin kinase inhibitor (TKI) - Sprycel or Glivec, had a low level of fluorescence anisotropy, median value and 95% CI for Hydroxyurea group 151 (137.1-158.6) vs TKI group 138 (124.4-147.8) p<0.04. No differences of fluorescence anisotropy was observed between the group of MPN patients who received JAK inhibitor (Jakavi) or Hydroxyurea. The CD42b expression is low in patients versus controls (median: 17.87% vs 94.16%, P<0.001), there is no difference in the CD42a value range (P=0.51). The CD61/CD41 expression (GP IIb-IIIa) presents also lower values in patients (median: CD 61= 98%; CD 41=91.13%) versus controls (median: CD 61=98%; CD41=93.17%), statistical significance obtained only for CD61 expression. Production of ROS is higher for patients with MPNs and CML patients compared with healthy controls. CML patients in acute or blastic phase have higher level of ROS production compared with patients in chronic phase (1.23 vs 1.09, p=0.03). Our results of anisotropy measurements did not reveal any influence of ROS in MF modifications (0.15 vs 0.13, without statistical significance) or with platelet receptor expression.

**Summary/Conclusions:** The presence of JAK 2 mutations in MPN patient is associated with a low fluidity of platelet membrane. Association of Anagrelide or TKI inhibitor is associated with lower level of fluorescence anisotropy. The fluidity of platelet membrane could be an important parameter which influenced the expression of platelet receptor. We have to observe in the future if this group with high level of fluorescence anisotropy had a high risk of thrombosis. All these results will be verified in a patients cohort and need to be checked any correlation between modification of fluidity membrane production ROS and expression of microparticles platelet derived.

**E1603**

**USE OF ROTATIONAL THROMBOELASTOGRAPHY TO PREDICT CENTRAL VENOUS CATHER RELATED VENOUS THROMBOSIS IN CHILDREN: PRELIMINARY RESULTS**

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**Background:** Central venous catheters (CVCs) have been widely used in hospitals. In children, however, CVCs are rarely replaced due to its small size. In pediatric age group exact risk factors for CVC related venous thrombosis have not been shown yet. Rotational thromboelastography (ROTEM®) measures clot formation and stability and clogulopathy. Aims: We aimed to predict CVC related venous thrombosis via ROTEM parameters in high risk group patients.

**Methods:** Study included patients who required CVC insertion due to any reason and who were not on any anticoagulation treatment during the week before the CVC insertion. On the day of CVC insertion clotting time (CT), clot formation time (CFT), maximum clot firmness (MCF), and alpha angle (AA) were measured for intrinsic (INTEM), extrinsic (EXTEM), and fibrinogen (FIBTEM) pathways via ROTEM. At one week of insertion and at removal of CVC, Doppler ultrasound imaging was performed to the vein that catheter was removed. Results: A total 14 patients were included in the study. Median age was 3.9 years (3-17.9 years). Ten (71%) of the patients had jugular vein, four (29%) patients had femoral CVC. Median duration until removal of CVC was 15.5 days (7-56). Thrombosis was detected in one patient (7%) at first week of CVC insertion (Patient 10). When the the ROTEM parameters were examined, this patient had lowest CT and highest AA in EXTEM, and the highest AA in INTEM, indicating moderate pro-coagulant status (Table 1). Also patient 14 had similar AA as patient 10 in EXTEM and INTEM but was not found to develop thrombosis by the 18th day of insertion. However, CVC of that patient wasn't removed yet.

**Table 1.**

<table>
<thead>
<tr>
<th>Patient</th>
<th>CT (sec)</th>
<th>CFT (sec)</th>
<th>MCF (mm)</th>
<th>AA (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>105</td>
<td>120</td>
<td>150</td>
<td>10</td>
</tr>
<tr>
<td>Patient 2</td>
<td>110</td>
<td>125</td>
<td>160</td>
<td>15</td>
</tr>
<tr>
<td>Patient 3</td>
<td>120</td>
<td>130</td>
<td>170</td>
<td>20</td>
</tr>
</tbody>
</table>

**Summary/Conclusions:** Nearly 2/3 of Khorana intermediate risk patients developed a PE while on antineoplastic treatment and inside this group over 50% were treated with well-recognized high thrombotic-risk drugs. The inclusion of antineoplastic drugs in a predictive thromboembolic model in oncologic patients could improve the benefit-risk of the use of LMWH prophylaxis in some patients without a high risk Khorana score but however at high risk of thrombosis. More prospective studies are needed to analyse the benefit of antithrombotic prophylaxis in oncologic patients receiving outpatient chemotherapy treatment.

**E1604**

**THE POTENTIAL ROLE OF ANTIINEOPLASTIC DRUGS IN THE PREDICTION OF THROMBOTIC RISK IN ONCOLOGIC PATIENTS IN ADDITION TO THE KHORANA SCORE**


1Hematology, Complejo Hospitalario de Navarra, Pamplona, Spain

**Background:** Venous thromboembolism (VTE) is common in patients with cancer. Several risk factors (related with patient, tumour and treatment) have been already identified. Thromboprophylaxis (TP) with low molecular weight heparin (LMWH) is associated with a reduction of symptomatic VTE but without clear benefit in survival as the number of major bleedings is increased. To primary TP in newly diagnosed cancer patients starting chemotherapy (CT), a risk assessment model (based on clinical and laboratory variables) was developed (the Khorana score). Many patients with intermediate risk (without thromboprophylaxis indication according to Khorana-based clinical guidelines) develop VTE episodes. Factors as tissue factor-bearing micro particles and D-Dimmer levels in addition to lepoidanilide, platin and gencitabine based therapies are associated with VTE high risk. Its efficacy as a predictive tool is a matter of debate.

**Aims:** This retrospective, observational study is aimed to assess the Khorana score efficacy in predicting the VTE risk and analyze some treatment related factors as predictive complementary tools.

**Methods:** We analyzed the demographic characteristic, the Khorana score and the antineoplastic treatment of oncologic patients diagnosed of pulmonary embolism (PE) from December 2010 until December 2016 at theComplejo Hospitalario de Navarra. At baseline, the Khorana score classified patients as low risk (0 points) intermediate risk (1-2 points) or high risk (≥3 points) for VTE.

**Results:** 102 oncologic patients were diagnosed of PE. Patient baseline characteristics are showed in table 1. In 27.5% (n=28) PE diagnosis preceded to cancer diagnosis, in 26.5% (n=27) PE occurred at least 1 month beyond the end of antineoplastic treatment and in 46.1% (n=47) PE was diagnosed during the treatment (chemotherapy +/- radiotherapy). In this last group the median time from the treatment beginning and EP diagnosis was 3 months (0-46). The stratification according to the Khorana score (at baseline) was: ‘low risk’ 21.3%, intermediate risk 61.7%, and high risk 17%. In the intermediate risk group (n=29) the drug-based therapy was: 44.8% platin (n=13), 6.9% gencitabine (n=2), 2.5% lepoidanilide (n=1) and 48.3% non-related-thromboembolic treatment (n=14). Most of cases (97.1%) were managed with LMWH (enoxaparin 1mg/kg/twice a day). Only 2 patients were treated with non-fractionated heparin and 1, enrolled in a clinical trial, was treated with direct oral anticoagulants.

**Table 1.**

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Khorana score</th>
<th>Therapy</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Appearance (mm)</th>
<th>Diagnosis (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>0</td>
<td>No</td>
<td>33</td>
<td>Male</td>
<td>No</td>
<td>19</td>
</tr>
<tr>
<td>Intermediate</td>
<td>2</td>
<td>Yes</td>
<td>56</td>
<td>Female</td>
<td>Yes</td>
<td>2</td>
</tr>
<tr>
<td>High</td>
<td>4</td>
<td>Yes</td>
<td>78</td>
<td>Male</td>
<td>Yes</td>
<td>45</td>
</tr>
</tbody>
</table>

**Summary/Conclusions:** In this study we reported our preliminary results. We detected thrombosis only in one patient and due to this limited sample size, we may suggest that CT and AA in EXTEM, and AA in INTEM prior to insertion of CVC may be predictive for catheter related thrombosis development. Such patients with pro-coagulant findings at ROTEM prior to CVC insertion may need prophylactic anti-coagulation. The results in a larger sample size will be more definitive to make a conclusion.
**Transfusion medicine**

**E1605**

CLINICAL OUTCOMES AND UTILIZATION OF BLOOD BANK RESOURCES OF PATIENTS WITH THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP), HEMOLYTIC UREMIC SYNDROME (HUS), AND OTHER MICROANGIOPATHY-ASSOCIATED THROMBOSIS SYNDROMES: A 14 YEARS' EXPERIENCE

C.-T. Lee1,2,*, C. Thow3, K.P. Lim3, S.T. Lim3, J. Mah3, N.N. Zhang3, M.L. Tung1, L.K. Tan1,3, S.Y. Lee1,2,3

1Department of Haematology-Oncology, National University Cancer Institute Singapore, 2Yong Loo Lin School of Medicine, National University Singapore, 3Blood Transfusion Service and Blood Donation Centre, Department of Laboratory Medicine, National University Hospital, Singapore, Singapore

**Background:** TTP, HUS and other thrombotic microangiopathy are rare, complex clinical syndromes which are characterized by thrombocytopenia, microangiopathic haemolytic anaemia, (MAHA) and systemic thrombosis. The introduction of plasma exchange (PEX) has dramatically reduced the mortality of these patients, and has become standard of treatment. Although the clinical outcome of these conditions is heterogenous, with multiple clinical complications and prolonged hospital stay, there is no previously published data to provide measurement of blood bank and hospital resource utilization associated with its clinical management.

**Aims:** We performed a retrospective cohort study of 42 consecutively treated patients with MAHA and analyzed their clinical and laboratory characteristics, treatment outcomes and plasma product utilization.

**Methods:** Medical records of these patients treated from 2002-2017 were reviewed. We used the standardized criteria based on the consensus on standardization of terminology in TTP to define clinical response. (Scully et al J Throm Haemost 2017).

**Results:** In our series, the causes and number (%) of MAHA were TTP-HUS (18, 42.9%), autoimmune disorder-associated MAHA (13, 31% i.e. 9 SLE and 4 Sjögren’s syndrome), cancer-related MAHA (4, 9.5%), drug-induced (3, 7.1%), post-transplant and infection-related microangiopathy (4, 9.5%). The average number of PEX sessions required to achieve overall clinical response in TTP, autoimmune-associated MAHA, HUS and drug-induced microangiopathy was 18.2±17.9, 11.5±7.6, 13.0±8.7 and 7.3±6.7, respectively. The mean follow up time was 40.8 months. 5 patients (11.6%) died during the course of treatment in index hospitalization, 12 (27.9%) were refractory to PEX and 24 patients (55.8%) responded to PEX, and 1 patient was lost to follow up. 1 patient relapsed 8 months after achieving clinical remission and was successfully treated with Vinristine. Another patient developed exacerbation and was palliated eventually. For the refractory cases, 7 patients were given Rituximab, 5 achieved clinical response while those who were given Vinristine (n=3) and Cyclophosphamide (n=2), achieved clinical response with a median of 15 days from the time second line agents were used. The 1 year overall survival of those who received second line treatment compared to patients who responded to only PEX and standard of care was 59% and 80% (p=0.51), respectively.

**Summary/Conclusions:** The clinical outcome in terms of survival in our cohort is in keeping with that of other registry and cohort (Hovinga et al Blood 2010). Our data which demonstrate the health care resource utilization show that management of these patients is expensive. While small in terms of incidence, it poses an economic burden disproportionate to its overall size.

**E1606**

HEPATITIS E VIRUS: INVESTIGATION IN NORTH ITALIAN BLOOD DONORS

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**Background:** Hepatitis E virus (HEV) is a major cause of acute hepatitis worldwide, with a high risk for transmission safety. Recent data from Europe showed a HEV IgG prevalences of 6.8% in German blood donors, 27% in Dutch blood donors, and 52% in an hyperendemic area in the South of France.

**Aims:** The aim of this study was to determine the prevalence of anti-HEV reactivity and HEV viremia in Italian blood donors, in order to estimate the risk of transmission.

**Methods:** Nearly 10,000 samples were collected from anonymized, unpaid donors at the “Lecco processing and validation blood center” (Lombardy, Italy) from June to July 2016. Samples were tested individually (individual-donation nuclease acid test [ID-NAT]) for HEV RNA using the Procleix HEV assay (95% limit of detection 7.9 IU/mL). Initial TMA-reactive samples were retested and considered positive if the retest result was reactive. For the serology study, a subset of 2000 donations was tested for HEV IgG using DiaPro HEV ELISA kit (Diagnostic BioprobesSrl, Milano, Italy). HEV IgG and IgM were analyzed in ID-NAT positive samples at the time of donation and in the follow up, collected one year after the index donation.

**Results:** The prevalence of IgG anti-HEV in north Italian blood donors was 7.4%. Nine out of 9,726 donor samples gave reactive values by the ID-NAT assay for HEV RNA. Among them, only one sample was confirmed to be reactive in additional TMA tests. None of the 9 HEV RNA initially reactive samples had circulating IgM or IgG antibodies against HEV. In follow up, only the repetitive reactive donor showed a IgM and IgG seroconversion, indicating primary HEV infection. Therefore, we estimated that the risk of receiving a potentially infectious blood unit is of 1:10,000 (upper bound of the 95% confidence interval, 1:1700).

**Summary/Conclusions:** This study confirms that viremic blood donation, although small, is not negligible. The clinical impact of HEV infection among blood recipients remains to be assessed. These data need to be considered when deciding a national policy for preventing HEV transmission.

**E1607**

SHORT-TERM ADMINISTRATION OF RECOMBINANT HUMAN ERYthropoietin decreases B cell in Human peripheral bLOOD

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1Department of Laboratory Sciences, Gunma University Graduate School of Health Sciences, 2Blood Transfusion Service, 3Department of Hematology, Gunma University Hospital, Maebashi, Gunma, Japan

**Background:** Erythropoietin (EPO) is hematopoietic factors participating in red blood cell production, and accelerates proliferation and inhibits apoptosis of erythroblasts. It is reported that EPO has pleiotropic effects including anti-apoptotic action for some cells, antioxidant action, vasculatization action, and promoting cell division in addition to stimulation of erythropoiesis as well, whereas there are conflicting results of small cohorts as to its effect on blood immune cells.

**Aims:** We analyzed peripheral white blood cell subsets in patients who received one bolus administration of recombinant human erythropoietin (HuEPO) to examine the effect of EPO on human immune system.

**Methods:** One hundred nineteen autologous blood donors (male/female 62/57) in Gunma University Hospital were enrolled in this study after written informed consent. All the patients had no infections or inflammation. Forty nine patients were treated with HuEPO (Epoetin alpha or Epoetin beta, 24,000 IU, respectively) once after blood donation because of low hemoglobin concentration and 70 were not treated. Peripheral blood samples were obtained at the time of the first phlebotomy and after 1 week from the same patient. We measured the number of WBC, lymphocytes, myeloid dendritic cells (mDC), plasmacytoid dendritic cells (pDC), CD4+ T cells, CD8+ T cells, Natural killer (NK) cells, B cells, monocytes, and neutrophils of peripheral blood before and after HuEPO administration by flow cytometry. All the patients had no infections or inflammation. Forty nine patients in treatment group, there was no significant change in any type of immune cell. In non treatment group, there was no significant change in any type of immune cell. There was no significant difference between treatment group and non treatment group. Paired and unpaired Student’s t-test were used to compare absolute counts and percentages of each cell, P values<0.05 were considered significant. This study was approved by the research ethics committee of our hospital.

**Summary:** The number and percentage of lymphocyte in WBC decreased significantly after HuEPO administration from 1885.0±520.8/µl to 1798.7±439.0/µl, in absolute number (p=0.019), and from 33.2±8.57% to 30.0±7.32% in percentage (p=0.023). The numbers of whole WBC, mDC, pDC, monocyte and neutrophil did not change significantly. In respect of lymphocyte subsets, absolute number of CD8+ T cell, NK cell and B cell significantly decreased from 358.9±257.0/µl to 311.5±210.9/µl (p<0.01), and from 290.6±157.6/µl to 16.5±13.6 /µl (p=0.045), respectively. Moreover, other B cell subsets, such as transitional B cells, memory B cells and marginal zone B cells, also showed a trend of decrease. However, percentages of naive B cell and IgD−CD27− B cell in total B cell did not change. These suggested that whole B cell decreased, not a specific subset of B cell. In non treatment group, there was no change of lymphocyte subsets.

**Summary/Conclusions:** These findings suggested that just one administration of rhEPO influenced human immune system, especially via reduction of B cell in peripheral blood, with unknown mechanism so far.
Background: At most centers, the majority of patients who request bloodless medicine are members of the Jehovah’s Witness (JW) faith. But, there are no standard, established guidelines to manage pancytopenia in these patients, nor are there many studies to inform optimal treatment approaches. The most troublesome patients who request bloodless medicines are patients with hematology malignancies. The treatments of these patients are considerable challenges. They have not only problems of severe pancytopenia, but also require intensive chemotherapy. Since 2000, our hospital has been a bloodless center. This study was retrospectively analyzed for hematologic malignancies and aplastic anemia with bloodless treatments in Soonchunhyang university hospital.

Aims: This study was retrospectively analyzed for hematologic malignancies and aplastic anemia with bloodless treatments in Soonchunhyang university hospital.

Methods: A retrospective review of medical records was performed of 44 patients with hematologic malignancies and aplastic anemia who request bloodless medicine from January 2006 to December 2015 at Soonchunhyang university hospital.

Results: Of 44 patients, 48% were men (n=21) and 52% were women (n=23). The median age of the study population at the time of diagnosis was 62 years (range 16-87). Thirteen patients (29.5%) were acute leukemia, 15 (34.1%) patients with non-Hodgkin’s lymphoma (NHL), 2 (4.5%) patients with aplastic anemia (AA), 6 (13.6%) patients with chronic myeloid leukemia (CML), 4 (9%) patients with myelodysplastic syndrome (MDS) and 4 (9%) patients with multiple myeloma (MM). Thirty one patients were treated with chemotherapies and 13 patients were treated with supportive care only. Among 44 patients 27 patients were died. Most common cause of attribution to death was anemia (92.5%). And Chief complaint at death was dyspnea (88%). Median survival of acute leukemia was 1 month (95% CI, 0.41-1.59).

Table 1.

Summary/Conclusions: In bloodless treatment, CML, MM and lymphoma had a relatively good prognosis. However, AML and MDS were showed a poor prognosis. Therefore, further studies are needed to improve survival for bloodless patients with hematologic malignancies.

E1609
PREOPERATIVE ANEMIA: A SINGLE INSTITUTION EXPERIENCE IN SPAIN
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Background: Preoperative anemia is considered as a strong predictor of postoperative red cell transfusions, and has also been linked to increased morbidity and mortality in surgical patients, but it is frequently overlooked.

Aims: The objective of this study is measure real impact of preoperative hematological assessment and optimization of anemic patients in terms of decreasing blood cells transfusions.

Methods: A retrospective study of patients undergoing elective surgery in subgroups of high or low risk of bleeding. All the patients were referred from pre-anesthesia consultation for performing a 4-week hematological protocol in order to optimize the hemoglobin level to a near normal value. We identified the underlying cause of anemia and offered the treatment according to the elology. The primary outcomes were the response to therapy defined as reaching the Hb level >13 g/dL or increasing of >2 g/dL from basal level, and the rate of blood transfusion.

Results: Mean age was 70.4 years, with a male-female ratio of 1:2, and the patients were divided into 2 groups according to the bleeding risk: high risk 74% (hip and knee replacement, cœstectomy, colonomy, maxilofacial surgery), and low 26% (mastectomy, gynecology or spine surgery), with a median hemoglobin of 10.9% and 10.1%, respectively. A diagnostic workup was performed in order to provide appropriate treatment: iron deficiency anemia (83.9%), anemia of chronic disease (10.3%), folate or vitamin B12 deficiency (5.8%). The patients with iron deficiency anemia received oral (62%) or intravenous iron (38%), and third of patients had to change from oral to intravenous iron by intolerance or poor response. The response to treatment was reached by 44.7% of patients, in an average time of 26.4 days. The rate of blood transfusion was 18% in good responders (0.5 packed red blood cells per patient) and 63% in poor responders (1.6 packed red blood cells per patient).

Summary/Conclusions: The treatment strategies during preoperative period, and the effort to reach a near to normal hemoglobin level, could minimize the amount of red blood cell transfusion the patients will be exposed in the postoperative period. Our data provide evidence about the effectiveness of a prompt evaluation and correction of preoperative anemia in a maximum time of 4 weeks.

E1610
RED BLOOD CELLS (RBC) AND PLATELET (PLT) TRANSFUSIONS IN TRANSPLANTED AND NOT-TRANSPLANTED PATIENTS WITH HEMATOLOGICAL MALIGNANCIES
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Background: Patients with hematological malignancies require often and prolonged hospitalisations during the course of their treatment, in part due to increased and frequent transfusion demands.

Aims: The objective of the study was to assess the factors affecting transfusion needs in a Hematology Department (bone marrow transplant unit- BMTU, post-transplant unit-PTU, hematologic clinic).

Methods: The patients that were hospitalized between 1/1/2015 and 31/12/2015 were analyzed. Data regarding the underlying disease, the disease status, type of transplant, duration of marrow aplasia and donor-patient blood group mismatch were obtained from the medical records. The analysis was restricted to the transfusion of packed RBCs and units. Differences between groups were assessed using non-parametric statistics (Kruskall-Wallis and Mann-Whitney U-test).

Results: There were 523 admissions of 256 different patients. Complete data for analysis could be obtained for 487 admissions of 237 patients (92.6% of patients, 93.1% of admissions), corresponding to 10,673 days of hospitalization. Total number of blood products transfused was 2284 packed RBC units, 13883 PLT units (apheresis platelets counted as 5 units). Values are reported as median (range), unless otherwise specified. In the BMTU, the type of transplant was correlated with transfusion needs: number of RBC units transfused per admission was 2 (1-5) for autologous transplanted (AUTO) patients, 4 (1-28) for allo-transplanted (ALLO) (no difference between sibling and matched unrelated donors), and 7 (1-14) for haplo-identical transplants (HAPLO), p<0.001. Platelet units requirements were respectively 15 (5-45) for AUTO, 20 (5-205) for ALLO and 50 (30-130) for HAPLO, p<0.001. The median number of days for apheresis was 19 (13-23) days in AUTO, 22 (16-44) in ALLO, 30 (29-40) days in HAPLO transplantation, p<0.001, while the duration of aplasia in days was 9 (4-19) in AUTO, 13 (5-32) in ALLO and 25 (20-38) in HAPLO, p<0.001. The longer duration of aplasia and hospitalization was correlated with greater transfusion needs. In the PTU there was no statistically significant difference in transfused RBC or PLT units with regard to transplant type. Disease status (response versus active disease) was only correlated with RBC units transfused in PTU [2 (1-29) vs 6 (1-56) units respectively, p=0.006]. Donor – patient blood group mismatch was correlated with increased transfusion demands in BMTU for RBCs [4 (1-28) vs 2 (1-5), p<0.001] and PLTs [25 vs 15, p<0.001]. In hematologic clinic, the underlying disease was correlated with transfusion needs in BMT and PLTs, as shown in table 1. Patients with AML had the higher needs in RBCs and PLTs, whereas patients with lymphoma had the lowest needs in RBC transfusions. Disease status was not correlated with transfusion needs. The duration of aplasia was correlated with the number of RBC units (Pearson’s r=0.66, p<0.001, r²=0.435) and of PLTs transfused (Pearson’s r=0.78, p<0.001, r²=0.61).

Table 1. Units transfused in hematology clinic

Summary/Conclusions: The main determinants of transfusion requirements are the duration of aplasia, the type of transplant and the disease, with myeloid malignancies requiring more transfusions. The establishment of haplo-identical transplantations has increased the transfusion needs due to longer period of aplasia.
Acute lymphoblastic leukaemia - Biology

PB1611

BOTANICAL ALKYL HYDROQUINONE HQ17(3) EXERTS CYTOTOXICITY TO T(9;22) PHILADELPHIA CHROMOSOME SUP-B15 ALL CELLS THROUGH INDUCING ENDOPLASMIC RETICULUM STRESS, AUTOPTOXY, AND TACIFICATION

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Background: Patients suffering from Acute lymphoblastic leukemias (ALLs) harboring t(9;22) genetic abnormality are classified very high risk (VHR) ALLs displaying poor clinical outcome irrespective of intensive chemotherapies and tyrosine kinase inhibitor treatment. Development of new adjuvant therapeutics will provide great value. HQ17(3)-induced nuclear translocation of AIF, in compatible with mitochondria disturbance and caspase-independent cell death thereafter.

Methods: Cell growth inhibition in response to HQ17(3) w/o inhibitors was analyzed by ACP assay. Cells were stained by Annexin V/PI and analyzed by flow cytometry for cell death. Lysosomal protease inhibitors (AEBSF (serine protease inh.), pepstatin/CA074-Me (cathepsin D/B inh.)) or autophagy inhibitors (Bafilomycin A1) were used in combination with HQ17(3) in some experiments. Acridine orange stain and confocal microscopy are used to visualize the changes of acidic vesicles. Autophagic flow in response to HQ17(3) was revealed by aggregation of ectopically expressed EGFP-LC3. Western blot analysis were used to repress the expression of Beclin-1. Nuclear accumulation of apoptosis inducing factor (AIF) was revealed by fluorescence microscopy.

Results: Enlarged acidic vesicles accumulated soon after HQ17(3) treatment, and diminished when cell death ensued. HQ17(3)-induced cell death could not be attributed to caspase release from the mitochondrial membrane permeabilization (LMP) as caspase inhibitors did not attenuate the cell death. HQ17(3) enhanced autophagy as revealed by aggregation of ectopically expressed EGFP-LC3. Inhibition of autophagy by Bafilomycin A1 or knockdown of the essential autophagy-related Beclin 1 by shRNA could partially attenuate HQ17(3)-induced cell death. Further, HQ17(3) treatment gave rise to early ER stress as revealed by enhancement of eIF2a phosphorylation and up-regulation of ER chaperone Grp78. HQ17(3) induced nuclear translocation of AIF, in compatible with mitochondria disturbance and caspase-independent cell death thereafter.

Summary/Conclusions: In Ph+-ALL SUP-B15 cells, HQ17(3) acts in multi-facet: a) lead to oxidative stress and perturb mitochondria membrane integrity, b) induce ER stress and calcium mobilization to mitochondria, c) induce BAX and caspase-dependent cell death, c) induce ER stress and calcium mobilization to mitochondria, cleave and release AIF to mediate nuclear chromatin cleavage, c) HQ17(3)-induced autophagy may be implicated cell death. This study shows agents that are capable of eliciting an intricate effector network in therapy-induced cytotoxicity will have potential as adjuvants controlling the VHR-Ph+-ALL cells refractory to conventional high dose chemotherapies and TKI regime.

PB1612

TARGETED MUTATIONAL PROFILING OF CHILDHOOD AND ADULT ACUTE LYMPHOBLASTIC LEUKAEMIA PATIENTS

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Background: Acute lymphoblastic leukaemia (ALL) is the most common cancer in children, representing about 80% of acute leukemias, whereas it is less common in adults (20%). Identification of cytogenetic aberrations and a small number of molecular abnormalities are still the most important risk and therapy stratification methods in clinical practice today.

Aims: The aim of the present study was to assess mutational profile of both childhood (cALL) and adult acute lymphoblastic leukaemia (aALL) patients, by applying targeted next generation sequencing (NGS) on MiSeq System.

Methods: We analyzed DNA samples from 34 de novo ALL patients (17 cALL and 17 aALL) using TruSeq Amplicon – Cancer Panel (TSACP) that targets mutational hotspots in 48 cancer related genes (212 amplicons). The bioinformatics analyses was conducted using processing pipeline composed of both freely available open source bioinformatics tools as well as tools developed in house. The average coverage of high-quality sequences was 2000 × per ampli- con. Ten genes were discarded due to insufficient coverage, therefore we ana- lyzed a total of 183 amplicons from 38 genes. Variants were identified in relation to the GRCh37 reference genome by applying a Bayesian approach and compared to public genetic variation databases and in-house databases.

Results: We identified a total of 331 (159 cALL, 172 aALL) variants in the coding regions (median per patient: 9, range: 6–12; median per cALL: 9; range: 6–12; median per aALL: 10; range: 7–12) and 429 (211 cALL, 218 aALL) variants in the non-coding regions (median per patient: 13 range: 10-15; median per cALL: 13; range: 10-14; median per aALL: 13; range: 9–11) variants. Overall, 98 variants (median per patient: 2.8, range: 1–6) were potentially protein-changing, including nonsense, frameshift, and missense (NFM) mutations. There were no significant differences in the number of NFM mutations between cALL (total 47, median per patient: 3, range: 1–5) and aALL patients (total 51, median per patient: 4, range: 1–5). Moreover, we identified 5 NFM mutations in STK11 gene, 3 in ABL1, RET KRAS and 2 in HNF1A, NRAS, and NOTCH1. Observed in individual patients detected mutations predominantly disrupted Ras/RKT pathway (STK11, KIT, MET, NRAS, KRAS, PTEN). Additionally, we identified 5 patients with the same mutation in HNF1-A gene coding for transcription factor, disrupting both Wnt and Notch signaling pathway. Notch pathway was disrupted in two patients in which detected variants affected NOTCH1 gene. HNF1A and NOTCH1 variants were mutually exclusive, while genes involved in Ras/RKT pathway exhibit a tendency of mutation accumulation.

Summary/Conclusions: Our targeted NGS study showed low number of recurrent mutations in both cALL and aALL patients. This suggests that there was a suspicion of a disseminated malignancy. She underwent an MRI scan which showed extensive changes in her spinal column and hips. Bone marrow biopsy revealed Acute Lymphoblastic Leukaemia (ALL). Paucity of diagnostic material restricted the cytogenetic analyses. G-banding showed 46,XX[14], with FISH demonstrating loss of FOXO1/13q14.11 [44%], gain of 4q27-4q32 [44%], 17p13.1 [44%], 13q14.3 [44%], 22q11.2 [44%], and 21q22.1 [44%]. Identification of 8q24.21 [9%], 10q11.21 [9%], and 19p13.11 [9%]. Overall, a total of 653 variants (median per patient: 2.8, range: 1–6) were potentially protein-changing, including nonsense, frameshift, and missense (NFM) mutations. There were no significant differences in the number of NFM mutations between cALL (total 47, median per patient: 3, range: 1–5) and aALL patients (total 51, median per patient: 4, range: 1–5). Moreover, we identified 5 NFM mutations in STK11 gene, 3 in ABL1, RET KRAS and 2 in HNF1A, NRAS, and NOTCH1. Observed in individual patients detected mutations predominantly disrupted Ras/RKT pathway (STK11, KIT, MET, NRAS, KRAS, PTEN). Additionally, we identified 5 patients with the same mutation in HNF1-A gene coding for transcription factor, disrupting both Wnt and Notch signaling pathway. Notch pathway was disrupted in two patients in which detected variants affected NOTCH1 gene. HNF1A and NOTCH1 variants were mutually exclusive, while genes involved in Ras/RKT pathway exhibit a tendency of mutation accumulation.

Background: A 7-year old girl presented with backache, leg pain and difficulty walking. She was referred to the local paediatric oncology service, as she was known with Li-Fraumeni Syndrome (LFS). Cancer types linked to this disorder include soft tissue sarcomas, osteosarcoma, breast cancer, brain cancer, leukaemia and adenocarcinoma. The patient’s mother had breast cancer twice, and both her (monoyogotic) twin sister and older sister had adrenal cortical carcinomas removed. The patient was born with left sided twinning defects (absent left kidney, malformation, absent left ear and ear canal, Arnold-Chiari malformation with spinal cord syrinx and hydrocephalus). At presentation there was a suspicion of a disseminated malignancy. She underwent an MRI scan which showed extensive changes in her spinal column and hips. Bone marrow biopsy revealed Acute Lymphoblastic Leukaemia (ALL). Paucity of diagnostic material restricted the cytogenetic analyses. G-banding showed 46,XX[14], with FISH demonstrating loss of FOXO1/13q14.11 [44%], gain of MYC/8q24.21 [9%], ET6S-RUNX1 NEGATIVE, gain of RUNX1/21q22.12 [21%] and BCR-ABL1 NEGATIVE. The patient was treated according to NCI risk criteria on the least intensive regimen of the UK ALL 2003 trial. She achieved morphological remission after a 3-drug induction, and successfully completed further treatment, including intensification/CNS directed phase, interim mainte- nance and 2 delayed intensification blocks. She completed 5 years of follow- up and was transferred to the Long Term Follow-Up Clinic, when she presented with hypercalcemia. Peripheral blood and bone marrow biopsy confirmed a diagnosis of ALL. Although the phenotype resembled the profile of the first pre- sentation of leukaemia, the genetic aberrations appeared incongruent.

Aims: Establish the origin of the second episode of ALL in a patient with known Li-Fraumeni Syndrome. As treatment and outcome for relapsed ALL in com- parison with a second, primary, ALL are completely different, this information was crucial to guide further management.

Methods: We set out to comprehensively characterise the second ALL, including conventional G-banding and fluorescence in situ hybridisation (FISH). The
acquired results were compared with those derived from the first ALL diagnosis. Results: The diagnostic analysis revealed cases 36 XX-2,-3,-4 dél(0q23)5q31-7,-12,-13,-14,-15,-16,-17(13)/46,XX(7). Extensive FISH analysis confirmed the diagnosis of low hypodiploid ALL. This result was in line with the reported association between TP53 gene mutations in 90% of cases of low hypodiploid ALL, with these mutations often present in normal cells. [Holmfeldt, Nat Gen, 2013] At first sight, this did not reconcile with the original cytogenetic analysis and suggested the occurrence of a second episode of ALL. In order to further characterise the diagnostic genetics, FISH probes were used on archived diagnostic slides. Careful selection of probes demonstrated that the original leukemic sample contained two co-existing clones – one low hypodiploid clone (with an identical pattern of loss of gain of chromosomes as the second ALL) and one clone resembling a doubled up/triploid low hypodiploid clone.

Summary/Conclusions: This case report demonstrates the value of in-depth genetic analyses to guide management of patients with ALL. This patient proceeded with re-induction according to our current relapsed therapy guidelines (R) after which she has shown complete remission status for 5 years with low intensity treatment and irregularly relapsed when most patients are told they are cured.

Since the original diagnosis of ALL in 2007, research has vastly improved our understanding of the biology and genetic landscape of ALL. This has facilitated risk stratification, improved outcome after treatment and identified novel drug targets. Genomic profiling of low hypodiploid ALL has identified oncogenic activation of Ras and phosphoinositide 3-kinase (PI3K) signalling conferring sensitivity to PI3K inhibitors, thus providing therapeutic avenues if conventional treatment were to fail.

**PB1614**

**IMMUNOLOGICAL CHARACTERIZATION OF PH+ ALL BONE MARROW ACCORDING TO PROTOCOL RALL-2009**

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**Background:** The treatment results in Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL) have improved significantly in the era of tyrosine kinase inhibitors (TKIs). However, many patients relapse despite having received intense treatments with initially favorable responses. TKI therapy is known to modulate the immune system, and it may play a critical role in keeping the leukemia under control. However, little is known about the status of the immune system in patients with Ph+ ALL. Especially with the emerging immunotherapies in sight, it is vital to chart the immunological landmarks that could help us direct the treatment towards a more personalized course.

**Aims:** To characterize the immunological microenvironment in Ph+ ALL bone marrow (BM) by multiplex immunohistochemistry (IHC).

**Methods:** Ph+ ALL BM biopsies from the diagnosis stage were collected from Helsinki University Hospital and Tampere University Hospital (N=31). BM biopsies from non-leukemic (NL) controls (N=14) were used as a reference. Samples were hematopathologically evaluated and a tissue microarray (TMA) was constructed by selecting two BM cores with high leukemic cell infiltration per patient. The TMA sections were stained with both fluorescent and chromogenic in situ hybridization (FISH) was performed for detection CDKN2A deletion, TEL/AML1, MLL rearrangement, MYC in situ hybridization (FISH) was performed for detection CDKN2A deletion, TEL/AML1, MLL rearrangement, MYC

**Results:** The CD4+/CD8+ ratio was lower in Ph+ ALL BM versus NL BM (1.3 [interquartile range (IQR) 1.0-1.9] vs 2.0 [IQR 1.7-2.4], p=0.0134) indicating that there are relatively more CD8+ T cells in the leukemic than in the non-leukemic bone marrow. The ratio of memory CD4+CD45RO+ T cells in Ph+ ALL BM versus NL BM was elevated (21.0% [IQR 16.7-28.5] vs 13.0% [IQR 8.7-15.9], p=0.0044). The difference in memory CD8+CD45RO+ T cells was not significant (p=0.36). Further analysis of the T cell phenotype showed increased proportion of both PD1-positive helper T cells and PD1-positive CD8+ T cells in Ph+ ALL BM versus NL BM (29.7% [IQR 17.5-40.1] vs 6.9% [IQR 5.7-8.9], of CD4+ cells, p=0.0001 and 28.8% [IQR 13.2-38.0] vs 14.9% [IQR 9.6-18.7], of CD8+ cells, p=0.0107). The ratio of OX40-positive helper T cells was also higher in Ph+ ALL BM (21.7% [IQR 21.6-33.25] vs 18.5% [IQR 14.8-
Results: The prevalence of the CDKN2A deletion in all studied population was 24.5% (27 cases). The frequency of homozygous deletions was 70% (in 19 cases), heterozygous deletion was 30% (in 8 cases). CDKN2A deletion was detected in 14 (52%) patients with precursor-B phenotype, in 11 cases (41%) with T-ALL and in 2 (7%) cases with biphenotypical ALL. Our study demonstrated that CDKN2A deletion had no significant association with age, sex, WBC counts, BM blasts, risk stratification groups, complete remission (CR) and relapse rate in B-cell ALL. We didn’t reveal any significant differences in OS, clinical and laboratory dates between groups of patients with homozygous and heterozygous deletion of the CDKN2A deletion. The analysis for T-ALL has detected that CDKN2A deletion was strongly associated with high WBC count (the median is 86×10^9/L, p=0.004), with high 3q26.3 (75%) hyperdiploidy (IML level (the median is 3092 E3, p=0.0004) and no associating with CR and relapse incidence was found. We didn’t revealed relationship between CDKN2A deletion and TEL/AML1 rearrangement, MYC translocation, TP53 mutation and IAMP2. CDKN2A deletion didn’t have statistically significant impact on outcome of patients. The five-year relapse rate of patients with and without deletion was 85% and 76% (p=0.33; DFS was 92% and 65% (p=0.07), respectively. OS for T-ALL patients with and without deletion was 90% and 80% (p=0.63); DFS was 100% and 82% (p=0.24, respectively. (Figure 1).

Summary/Conclusions: We were unable to demonstrate prognostic value of the CDKN2A deletion in adult ALL patients and did not find significant associating between deletion of the CDKN2A gene and with known cytogenetic prognostic factors. However patients with T-cell ALL and CDKN2A deletion had a more aggressive clinical profile (features of high level WBC and LDH), but it didn’t associate with poor outcomes including overall survival.

Deletion of CDKN2A is not adverse prognostic factor in adult ALL treated according to protocol RALL-2009.

PB1616 FREQUENCY AND CLINICAL IMPACT OF CDKN2A/B GENE LOCUS IN AN ADULT T-ALL COHORT OF PATIENTS ENROLLED IN THE SPANISH PETHEMA GROUP PROTOCOLS


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Background: Recurrent 9p21 deletions involving CDKN2A/CDKN2B locus are frequent in ALL. The very few data regarding their prognostic significance in adult T-ALL have shown that homozygous deletions of the CDKN2A/CDKN2B locus are associated with improved overall survival (OS).

Aims: We precisely characterized the copy number status (CNA) of CDKN2A/CDKN2B locus by discriminating deletions in A or B gene in order to elucidate its clinical impact separately.

Methods: Samples from 30 adult T-ALL cases included in high-risk protocols of the PETHEMA group were analyzed by CytoScan array (Affymetrix). Additionally, we set up a genomic qPCR to screen for CDKN2A and CDKN2B deletions in all the cases analyzed. With that, we ask for clinical implications of CDKN2A/B locus in adult T-ALL patients.

Results: qPCR results showed that most of the 9p21 losses corresponded to homozygous deletions in both genes (36%, 10/28), while heterozygous deletions corresponded to 5.7% (3/53) and different CNA status between both genes (19/53), while heterozygosis deletion was prevailing in 30% (16/53) of the samples. Globally alterations in CDKN2A/B locus were detected in 70% of adult T-ALL patients. Different CNA status was found for CDKN2A and CDKN2B. Although homozygous deletion in CDKN2B was associated with a trend for better OS, the level of MRD was the only prognostic factor for OS in these cases.

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PB1617 BUTEIN KILLS ACUTE LYMPHOBlastic LEUKemia CELLS IN VITRO AND IN VIVO THROUGH FOXO3A AND CASPASE-DEPENDENT APOPTOTIC PATHWAYS

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Background: Acute lymphoblastic leukemia (ALL) is a common hematological malignancy in children. Discovering and developing effective chemotherapeutic drugs are needed for ALL.

Aims: In this study, the anti-leukemic effect and the potential molecular mechanisms of butein on ALL were investigated.

Methods: We examined the rate of apoptosis of CEM-C7 (T-ALL), CEM-C1 (T-ALL), MOLT-4 (B-ALL), RS4-11 (B-ALL) cell lines and primary ALL blasts from children with acute lymphoblastic leukemia (ALL). We tested the expression of the caspase-9, poly ADP-ribose polymerase (PARP), nuclear Forkhead class box O3a (FOXO3a) and BCL-2 interacting mediator of cell death (BIM) using western blot assay. We also analyzed the xenograft mouse model to examine the anti-leukemic effect of butein in vivo. Moreover, we showed that FOXO3a knockdown significantly decreased the apoptosis of butein, whereas overexpression of FOXO3a enhanced the butein-induced apoptosis. However, overexpression of FOXO3a mutation (C-terminally truncated FOXO3a DNA-binding domain) decreased the apoptosis by butein through decreasing the expression of BIM. Furthermore, treatment with butein was highly efficacious in vivo, with enhanced reduction of tumor burden in a xenograft model of ALL.

Summary/Conclusions: Our results therefore demonstrate the therapeutic potential of butein for ALL via FOXO3a and caspase-dependent apoptotic pathways.

PB1618 GENOMIC LANDSCAPE AT DIAGNOSIS AND RELAPSE IN CHILDHOOD ACUTE LYMPHOBlastic LEUKemia

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Background: Childhood acute lymphoblastic leukemia (ALL) is the most common of pediatric malignancies, but intensive chemotherapy now allows to obtain complete remission in over 90% of the cases. Nevertheless, 1 out of 5 children relapse.

Aims: In order to identify new markers prognostic of relapse, we analyzed SNP arrays of paired diagnosis and relapse samples from 8 B-ALL children.

Methods: The cohort included 3 males and 5 females, aged between 6 months and 21 years old (median age 4 years old). Bone marrow samples were analyzed for previously selected 337,069 single nucleotide polymorphisms (SNP) performed by Affymetrix. SNP array analysis was performed using Illumina Infinium HumanOmniExpress BeadChip (Illumina). We performed a supervised classification of the patients using a random forest model, and a genetic algorithm to select the most relevant SNPs. We used a Bonferroni correction to adjust for multiple comparisons. We compared the genomic landscape at diagnosis and relapse.

Results: The genetic landscape of diagnosis and relapse was highly conserved in 7 of the 8 cases, with only minor changes observed. However, the genomic landscape at relapse was different in the 8th case, with the addition of several novel genetic alterations. These alterations were associated with poor outcomes, including overall survival (OS) and event-free survival (EFS).

Summary: Our results suggest that the genomic landscape at relapse can be used as a predictor of treatment outcome and may identify novel therapeutic targets for ALL.
over, a (4;8) translocation was found to be more complex with 7 and 8 CNA on chromosomes 4 and 8. Patients with the most CNA and LOH also had a complex karyotype. Anomalies were observed in hot spot regions in 8p (encompassing CDKN2A/2B, PAX5 and JAK2) for 5 patients and 12p (including ETV6) for 3. Stable CNA were observed in the JAK/STAT pathway in 2 patients (JAK2) and LOH in the RAS/MAPK pathway (NRAS) in 1. Using the genetic classification of Moorman et al based on SNP array for 8 genes at diagnosis (IKZF1, CDKN2A/2B, PAR 1, BTG1, EBFC, PAX5, ETV6 and RB1), SNP reclassified our patients in 3 of good prognosis and 5 of poor prognosis, with a median of 2 CNA for the 8 genes of interest. The 2 patients with cytogenetic intermediate prognosis would thus probably have been considered for a more intense therapeutic regimen, i.e. allogeneic stem-cell transplantation. Moreover, SNP showed that 2 patients acquired an IKZF1 deletion, also of poor prognosis, while none of the children had TP53 mutation at diagnosis nor relapse.

Summary/Conclusions: SNP array allowed to identify additional anomalies (compared to karyotype) in all children tested and changed the prognostic value of diagnostic anomalies. Moreover, the identification of anomalies in the JAK/STAT pathway could indicate a treatment by tyrosine kinase inhibitors, which would possibly have positively modified outcome. Taken together, this new technology combined with classical analyses at diagnosis might modify therapeutic options in childhood ALL, especially in the subgroup with a normal karyotype.

PB1619
SCREENING OF NUDT15 GENE VARIANTS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA
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Background: In cells, while DNA bases can be protected by double helix formation and nucleosome packaging, deoxyribonucleotides triphosphates are unproctected, thus, are vulnerable to damage. One of the enzymes which are responsible for removing damaged nucleotides is Nudix hydrolase15 (NUDT15). NUDT15 works as a negative regulator in thiopurine metabolism. Thioguanines are active metabolites of thiopurines. Mechanisms of action of thioguanines are possible for removing damaged nucleotides is Nudix hydrolase15 (NUDT15). We claimed that, besides TPMT variants in Japanese patients, there might be other genetic abnormalities in NUDT15.

Aims: The aim of this study was to retrospectively and prospectively analyze bone marrow cells of children with T-ALL, to determine a frequency of recurrent cryptic chromosomal aberrations and to assess their impact on event free (EFS) and overall survival (OS).

Methods: Bone marrow cells of all patients were analyzed at the time of diagnosis by combination of conventional and molecular cytogenetic methods. For detection of the most frequent known chromosomal changes, i.e. rearrangements of TRC loci (TRA-14q11, TRB-7q34, TRG-7p14) and TLX3 gene (5q35), deletion of CDKN2A (9p21) and amplification of ABL1 (9q34), interphase FISH with locus-specific probes (Dako, Abbott Molecular) was used. Complex chromosomal rearrangements were proved by multicolor FISH and multicolor banding (24X/Gy/Cy3/Cy5 Probe Kit; MetaSystems) or CGH-SNP array (SurePrint G3 CGH+SNP 4×180K Agilent). For OS and EFS Kaplan-Meier analysis with Mantel Cox test was done.

Results: During the years 1996-2016 we examined archival material of 64 children with T-ALL (19 girls and 45 boys, median age 8.25 years). In total, chromosomal aberrations were detected in 86% of patients. The most frequent aberration was deletion of CDKN2A gene, which was found in 35/64 patients (19x homozygous, 16x heterozygous). Rearrangements of TRC loci were detected in 17/64 children (11x TRA, 6x TRB). TLX3 gene rearrangement was established in 15/64 patients. No aberration of TRG gene and amplification of ABL1 were found. Complex chromosomal aberrations were proved in 12/64 patients. In two cases, isochromosome of the long arm of chromosome 9 was found. 48 patients are living in the first/second complete remission. Relapse of the disease occurred in 17 patients, 16 children died. Best outcome (EFS and OS) was associated with TRA translocations (p<0.05). Patients with TLX3 rearrangement had significantly shorter OS and EFS (p<0.05).

Summary/Conclusions: Using molecular cytogenetic methods cryptic recurrent aberrations were proved in vast majority of patients. Rearrangement of TLX3 gene was related to poor outcome in contrast to TRA translocations associated with more favorable course of the disease. Our work attempts to clear up the significance of chromosomal aberrations related to childhood T-ALL in order to facilitate the patients’ stratification into cytogenetic prognostic groups and to identify patients at an increased risk of relapse similarly like it has been adopted in p8-ALL.

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PB1620
COMPREHENSIVE MOLECULAR CYTOGENETIC ANALYSES OF BONE MARROW CELLS IN 64 CHILDREN WITH T-ALL REVEALED PROGNOSTICALLY RELEVANT RECURRENT FINDINGS
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Background: T-ALL represents 15% of newly diagnosed children with ALL and it is clinically and genetically heterogeneous disease. Despite the use of intensive chemotherapy, relapse occurs in almost 25% of patients whose outcome remains dismal. Visible chromosomal aberrations are seen in approximately half of the cases, while cytogenetically cryptic aberrations are observed in almost all cases of T-ALL. However, prognostic implication of majority of them still remains unclear.

Aims: The aim of this study was to retrospectively and prospectively analyze bone marrow cells of children with T-ALL, to determine a frequency of recurrent cryptic chromosomal aberrations and to assess their impact on event free (EFS) and overall survival (OS).

Methods: Bone marrow cells of all patients were analyzed at the time of diagnosis by combination of conventional and molecular cytogenetic methods. For detection of the most frequent known chromosomal changes, i.e. rearrangements of TRC loci (TRA-14q11, TRB-7q34, TRG-7p14) and TLX3 gene (5q35), deletion of CDKN2A (9p21) and amplification of ABL1 (9q34), interphase FISH with locus-specific probes (Dako, Abbott Molecular) was used. Complex chromosomal rearrangements were proved by multicolor FISH and multicolor banding (24X/Gy/Cy3/Cy5 Probe Kit; MetaSystems) or CGH-SNP array (SurePrint G3 CGH+SNP 4×180K Agilent). For OS and EFS Kaplan-Meier analysis with Mantel Cox test was done.

Results: During the years 1996-2016 we examined archival material of 64 children with T-ALL (19 girls and 45 boys, median age 8.25 years). In total, chromosomal aberrations were detected in 86% of patients. The most frequent aberration was deletion of CDKN2A gene, which was found in 35/64 patients (19x homozygous, 16x heterozygous). Rearrangements of TRC loci were detected in 17/64 children (11x TRA, 6x TRB). TLX3 gene rearrangement was established in 15/64 patients. No aberration of TRG gene and amplification of ABL1 were found. Complex chromosomal aberrations were proved in 12/64 children. In two cases, isochromosome of the long arm of chromosome 9 was found. 48 patients are living in the first/second complete remission. Relapse of the disease occurred in 17 patients, 16 children died. Best outcome (EFS and OS) was associated with TRA translocations (p<0.05). Patients with TLX3 rearrangement had significantly shorter OS and EFS (p<0.05).

Summary/Conclusions: Using molecular cytogenetic methods cryptic recurrent aberrations were proved in vast majority of patients. Rearrangement of TLX3 gene was related to poor outcome in contrast to TRA translocations associated with more favorable course of the disease. Our work attempts to clear up the significance of chromosomal aberrations related to childhood T-ALL in order to facilitate the patients’ stratification into cytogenetic prognostic groups and to identify patients at an increased risk of relapse similarly like it has been adopted in p8-ALL.

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microenvironment. This powerful tool provides the baseline for further experiments like preclinical treatment trials or biology studies. While good engratment rates were published for primary pediatric ALL samples, engratment rates of adult ALL samples might be inferior, but remain largely elusive.

Aims: This study aimed to determine engratment and growing ability of primary adult ALL samples in immunodeficient mice. Genetic engineering was performed to evaluate transcription efficiencies by lentiviruses in PDX ALL cells.

Methods: Primary adult ALL and AML samples were transplanted into NSG mice in the absence of total body irradiation. Both frozen as well as fresh patient material was used. Human CD45 and human CD38 were stained in blood to monitor successful engratment. Mice were sacrificed before coming down with leukemia. Isolated cells from bone marrow and spleen were analyzed by flow cytometry. Genetic engineering was performed using lentiviral vectors systems and monitored by expression of fluorochrome markers and flow cytometry. Results: Engratment and growth was successful in NSG mice in 12 out of 15 primary adult ALL samples. Frozen samples showed a longer median engratment time. However, n=3 samples could already be isolated with an average time of 75.29 days. Generally, the engratment time varied from 47 days up to 166 days and was shortened for slow samples over several passages. Genetic engineering was successfully performed using lentiviral transduction to introduce expression of fluorescent colours for cell marking and monitoring in further experiments. Lentiviral transduction was performed in 8 ALL samples with BCR-ABL rearrangement and 2 ALL-AF4 ALL samples. Adult ALL PDX samples with chromosomal translocations showed very low transcription rates around 1%. Three AML samples with MLL-AF6, MLL-AF9 and MLL-AF10 translocation were analysed for this study. Interestingly and in contrast to ALL, transcription efficiency for AML rearranged samples was high with up to 60%. These values are similar to non-rearranged ALL samples having transcription rates between 30% up to 80%.

Summary/Conclusions: In summary, we observed a high engratment rate of primary adult ALL samples in immunodeficient mice which was above what we anticipated from the literature. These findings could already be isolated with an average time of 75.29 days. Generally, the engratment time varied from 47 days up to 166 days and was shortened for slow samples over several passages. Genetic engineering was successfully performed using lentiviral transduction to introduce expression of fluorescent colours for cell marking and monitoring in further experiments. Lentiviral transduction was performed in 8 ALL samples with BCR-ABL rearrangement and 2 ALL-AF4 ALL samples. Adult ALL PDX samples with chromosomal translocations showed very low transcription rates around 1%. Three AML samples with MLL-AF6, MLL-AF9 and MLL-AF10 translocation were analysed for this study. Interestingly and in contrast to ALL, transcription efficiency for AML rearranged samples was high with up to 60%. These values are similar to non-rearranged ALL samples having transcription rates between 30% up to 80%.

PB1622
SYNERGIC CHEMOTHERAPEUTIC EFFECT OF MENADIONE COMBINED WITH EPIGALLOCATECHINE-3-GALLATE OR DOXORUBICIN IN A HUMAN CELLULAR MODEL FOR ACUTE LYMPHOCYTIC LEUKEMIA

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Background: Epigallocatechine-3-gallate (EGCG) and menadione (vitamin K3, MD) are known as potent apoptotogens in cellular models for acute lymphoblastic leukemia (ALL) - Jurkat T cells.

Aims: The goal of this study was to explore the chemotherapeutic potential of MD combined with EGCG or DOX, and to determine whether there is a synergic interaction between these agents that could significantly enhance their antitu- morial activity in a synergic manner.

Methods: In order to determine the synergic potential of MD combined with EGCG or DOX, we performed clonogenic, clonogenic survival assays, and flow cytometry. Genetic engineering was performed using lentiviral vector systems and monitored by expression of fluorochrome markers and flow cytometry.

Results: Engratment and growth was successful in NSG mice in 12 out of 15 primary adult ALL samples. Frozen samples showed a longer median engratment time. However, n=3 samples could already be isolated with an average time of 75.29 days. Generally, the engratment time varied from 47 days up to 166 days and was shortened for slow samples over several passages. Genetic engineering was successfully performed using lentiviral transduction to introduce expression of fluorescent colours for cell marking and monitoring in further experiments. Lentiviral transduction was performed in 8 ALL samples with BCR-ABL rearrangement and 2 ALL-AF4 ALL samples. Adult ALL PDX samples with chromosomal translocations showed very low transcription rates around 1%. Three AML samples with MLL-AF6, MLL-AF9 and MLL-AF10 translocation were analysed for this study. Interestingly and in contrast to ALL, transcription efficiency for AML rearranged samples was high with up to 60%. These values are similar to non-rearranged ALL samples having transcription rates between 30% up to 80%.

Summary/Conclusions: In summary, we observed a high engratment rate of primary adult ALL samples in immunodeficient mice which was above what we anticipated from the literature. These findings could already be isolated with an average time of 75.29 days. Generally, the engratment time varied from 47 days up to 166 days and was shortened for slow samples over several passages. Genetic engineering was successfully performed using lentiviral transduction to introduce expression of fluorescent colours for cell marking and monitoring in further experiments. Lentiviral transduction was performed in 8 ALL samples with BCR-ABL rearrangement and 2 ALL-AF4 ALL samples. Adult ALL PDX samples with chromosomal translocations showed very low transcription rates around 1%. Three AML samples with MLL-AF6, MLL-AF9 and MLL-AF10 translocation were analysed for this study. Interestingly and in contrast to ALL, transcription efficiency for AML rearranged samples was high with up to 60%. These values are similar to non-rearranged ALL samples having transcription rates between 30% up to 80%.

PB1624
NATURAL HISTORY OF SECONDARY MULTICLONE PROLIFERATION WITH MYCOSIS FUNGOIDES 7 FOLLOWING TREATMENT OF RELAPSED ACUTE LYMPHOCYTIC LEUKEMIA

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Background: Approximately 90% of children with acute lymphoblastic leukemia (ALL) are cured with current treatment protocols. However, 15-20% of the patients still experience disease relapse. Most childhood ALL samples might be inferior, but remain largely elusive.

Aims: We set out to find pre B-ALL cell lines with DUOX translocation and ERG deletion as potential model systems for this novel subtype of pre B-ALL.

Methods: We screened a panel of ALL cell lines for aberrant expression of DUOX and ERG. Results: High levels of DUOX transcript with alternative exon 6 was present in cell line SUP-B15. However, cell line SUP-B15 did not express DUOX protein and consequently also not alternative ERG exon 6 transcript. These results indicate that focal ERG deletions are not a safe indicator for aberrant expression of DUOX. Cell line NALM-6 is presented as model system for DUOX/ERG pre-B-ALL.

References
2. Zhang Y, McCastlin K, Vidiashirin I. DUOX translocations. Genomic PCR was performed to detect focal ERG deletions. qRT-PCR showed expression of alternative ERG exon 6, transcriptional target of DUOX.
3. Genomic PCR showed that 2/66 pre B-ALL cell lines (NALM-6, SUP-B15) tested carried deletions targeting ERG exon 5. Results of DUOX qRT-PCR (Taqman probe Hs03037979_g1) were surprisingly inconsistent with Westerm blot analysis - which could only in part be explained by DUOX being a one exon gene. NALM-6 was the only cell line expressing the DUOX protein. Likewise, the alternative ERG transcript with alternative exon 6 was observed in SUP-B15 only.

Summary/Conclusions: In conclusion, focal ERG deletions in pre B-ALL cell lines (2/66) occur at similar frequencies as in the primary tumor. Cell line NALM-6 carries the DUOX-4HG translocation, expresses the DUOX protein and an ERG mRNA variant including the alternative exon 6. ERG deletions were present in cell lines NALM-6 and SUP-B15. However, cell line SUP-B15 did not express DUOX protein and consequently also not alternative ERG exon 6 transcript. These results indicate that focal ERG deletions are not a safe indicator for aberrant expression of DUOX. Cell line NALM-6 is presented as model system for DUOX/ERG pre-B-ALL.
Results: AMT aspirate morphology showed 5% of blasts. However, detailed 8-color flow cytometry according to the EuroFlow protocol revealed no cells with BCP-ALL-specific immunophenotype, but several subsets of BCP with aberrant CD19 (8.1%) and positive CD56 expression (2.1%). After cessation of maintenance therapy in 02-2016, continuous progression of infiltration was observed. Subsequent AMT aspirates revealed increasing proliferation of five different cell populations which show rare, aberrant immunophenotype. Three of them represented immature BCP: B1 (CD19+/CD10+dim/CD20+/CD22+/CD38+/CD45+/CD117+/HDL-DR+/SSCintermediate), B2 (CD34+/CD19+/CD10+hegemonous/CD20-/nTDT+/++/CD22+/CD33+/CD117+/CD123-CD45-DR+/++/SSClow), and B3 (CD34-/CD19-dim/CD10-dim/CD20-dim). The fourth population corresponded to non-lymphoid/non-dendritic cell precursors (CD34+/CD19+/CD10+/CD123+/CD45+/CD117+/HDL-DR+/SSCintermediate) and the fifth population showed the features of plasmacytoid dendritic cell precursors with aberrant CD10 expression (CD34+dim/CD19+dim/CD20+/CD20-/nTDT-/CD22+/CD38+/CD117+/CD123+/HLA-DR+/SSCintermediate). No statistical significant differences were observed regarding CXCL12 polymorphism in ALL cases compared to controls. Furthermore, no significant differences were observed among children with ALL compared to controls, whereas no differences were observed among children with ALL and healthy groups using logistic regression analysis.
Background: Intrachromosomal amplification of chromosome 21 (iAMP21) defines a rare subtype of pediatric acute lymphoblastic leukemia (pALL) occurring in approximately 2-3% of cases. The patients are older (median age is 9 years), usually have low white blood cell counts and show high relapse risk with standard therapy. Thus, it has been proposed to include ALL with iAMP21 as a distinct entity in the WHO classification of hematological malignancies.

Aims: To assess the frequency as well as the clinicopathological and genetic characteristics of ALL with iAMP21 in one of the three national diagnostic centers of pALL in Hungary. We sought to determine additional genetic aberrations associated with this rare entity.

Methods: Between 2008-2016, 175 samples of pALL patients were tested with FISH for BCR-ABL1, ETV6-RUNX1 and MLL translocations. When available, bone marrow karyotyping was used to verify the abnormal results. In one case with iAMP21, multiplex ligation-dependent probe amplification (MLPA) was used to verify the cytogenetic aberrations as well as to detect associated copy number alterations.

Results: Among the 175 samples screened with FISH, three showed evidence of iAMP21 (1.7%). Case 1 was a 16-year-old male who presented with thrombocytopenia and hepatosplenomegaly. Flow cytometry (FCM) showed common ALL phenotype with the expression of CD13 and CD33. FISH showed >10 RUNX1 signals in clusters in leukemic blasts, while karyotyping demonstrated r(21) with 7q deletion and +X. The lesions were verified by MLPA, which additionally revealed biallelic CDKN2B and RBL1 deletions. The patient was treated with ALL-IC BFM 2002 standard risk protocol. Following remission, isolated meningeal relapse occurred, for which he received radiotherapy. The patient died with recurrent meningeal disease without bone marrow involvement after 52 months. Case 2 was an 11-year-old girl, who presented with symptoms suggesting osteomyelitis of the tibia with unremarkable blood count. MRI showed multiple lesions in vertebrae as well as meningeal involvement of the spinal cord. Bone marrow biopsy and biopsy of the left tibia showed diffuse infiltration of lymphoblasts with only 5% leukemic cells in bone marrow aspirates. FISH detected 6-8 copies of RUNX1 in leukemic blasts, while karyotyping yielded only normal bone marrow cells. She was commenced on ALL-IC BFM 2002 standard risk and was later switched to high risk protocol. She is in complete remission after 14 months. Case 3 was an 11-year-old boy who presented with anemia and thrombocytopenia. FCM showed ALL with common phenotype with two populations; one being strong CD19+/CD66c+ and one with dim CD19+/CD66c-. FISH showed >10 RUNX1 signals in clusters in 95% of cells, while 52% showed BCR-ABL1 positivity. Bone marrow karyotyping yielded metaphases of poor quality (Figure 1).

Figure 1.

Summary/Conclusions: ALL with iAMP21 is a rare subtype with distinct clinicopathological characteristics. Presenting with only mildly elevated WBC in older children is typical, relapses are frequent if standard risk chemotherapy is administered. Association with BCR-ABL1 translocation is rare, having been reported so far in only 4 cases. Observing BCR-ABL1 translocation in a subpopulation of leukemic cells is an intriguing phenomenon; it indicates that this translocation may occur as a secondary event even after leukemic transformation has commenced.
Acute lymphoblastic leukemia - Clinical

PB1629

COMPLETE REMISSION WITH BLINATUMOMAB IN TWO PATIENTS WITH SKIN RELAPSED B-CELL ACUTE LEUKEMIA

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Background: Blinatumomab is a bispecific T cell–engager (BiTE) antibody (CD19/CD3) indicated in relapsed/refractory B-cell Acute Lymphoid Leukemia (r/r ALL) (Topp et al.). Extra-medulillary relapse is a rare event occurring in only 8% of the patients, of whom only 1.4% present a skin relapse which harbor a dismal prognosis (Gokbuget et al.).

Aims: Herein, we report the efficacy of Blinatumomab in two patients presenting with extra-medulillary relapse of ALL.

Methods: The first patient (a 40-year-old man) was diagnosed a CD19+ Ph - B-ALL in 2009. He received a chemotherapy regimen according to the GRAALL protocol (Huguet et al.) until complete remission (CR). In 2015, he was presented with a maculopapular rash of the right leg and the left flank, and two enlarged inguinal lymph nodes. Cutaneous relapse was attested by examination of skin biopsy specimen showing a blastic dermal infiltration harboring a CD10+, Tdt+ phenotype. The second patient was a 50-year-old male who presented, in 2016, a CD19+ B-ALL Ph+ Ikaros- without central nervous system involvement. He obtained a first CR after GRAALL induction with negative MRD (IgH) but he relapsed 3 months later with a maculopapular rash of his chest. The skin biopsy revealed a blastic dermal infiltration. These two patients with skin relapse received the same chemotherapy regimen (COPRAALL 2007 regimen) (Domenach et al.), with no efficacy (cutaneous blastic infiltrate). Both patients received one cycle of Blinatumomab from day 1 to day 28, at 28 μg per day, in an attempt to achieve CR before allogeneic stem cell transplantation, as previously described.

Results: At day 5 of Blinatumomab, an important non pruritic maculo-papular rash occurred in both patient, in the same area of the initial cutaneous involvement. Interestingly, it decreased after day 8. No new drug introduction or infection (bacterial, viral or parasitic) was documented in the days preceding or during Blinatumomab infusion. A skin biopsy performed at day 6 of Blinatumomab showed a prominent dermal CD3+ lymphocytic infiltrate with a perivascular, but also a peri-nervous distribution (on the first patient’s specimen only). Few lymphocytes marginated at the basement membrane and rare basal necrotic keratinocytes were also noted but without blast for the first, although few residual blast cells were observed on the second’s. One month later, another skin biopsy showed a CR with a lymphocytic infiltrate. The medullar CR was confirmed at the molecular level (MRD negative). The first patient received allogenic stem cell transplantation (SCT) from a matched related donor one month later. He presented an acute and chronic GVHD, and is now in complete remission with a follow-up of 7 months. The second is still waiting for a SCT.

Summary/Conclusions: These observations confirm the strong efficacy of Blinatumomab in r/r B-ALL. We observed a T-cell dermal recruitment 6 days after Blinatumomab initiation clinically mimicking skin GVHD. However, we couldn’t find specific histological features of GVHD, but only an “inflammatory dermal reaction”. The efficacy of Blinatumomab with cutaneous infiltration suggests promising activity in extra-medulillary relapse. Further studies are required to confirm a Blinatumomab-based strategy in extra medulillary relapsed B-ALL. This may provide a better understanding of how cytolytic synapses between T lymphocytes and intradermal blasts happen and the underlying homing mechanisms involved.

PB1630

A NOVEL METHOD FOR MINIMAL RESIDUAL DISEASE ANALYSIS IN PHILADELPHIA-NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIA: MODIFIED BIOMED-2 POLYMERASE CHAIN REACTION FOR IMMUNOLOGINULIN HEAVY CHAIN REARRANGEMENT

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Background: Recent studies have demonstrated the clinical importance of minimal residual disease (MRD) monitoring in adult acute lymphoblastic leukemia (ALL) as well as pediatric ALL. However, patient-specific polymerase chain reaction (PCR)-based MRD assessment, one of the most commonly recognized methods, is not widely used in clinical practice because it is expensive, time consuming, and technically difficult. Therefore, we modified the BIOMED-2 protocol, PCR for immunoglobulin heavy chain (IgH) rearrangement, to assess MRD in ALL easily and readily in our hospital.

Aims: The aim of this study was to examine the clinical utility of monitoring MRD by the modified BIOMED-2 PCR for IgH rearrangement in patients with Philadelphia-negative (Ph-) ALL.

Methods: We enrolled 54 patients diagnosed with Ph- ALL between 2006 and 2016 in our hospital. IgH rearrangement was detected in 35 patients using the standard BIOMED-2 PCR protocol. Patients who received palliative chemotherapy, never achieved remission (blasts >5%), or had no follow-up MRD data were excluded. Finally, data from 27 patients with Ph- ALL were analyzed. We assessed MRD with the modified BIOMED-2 PCR for IgH using bone marrow samples collected after each chemotherapy session. Patients’ MRD statuses were classified as follows: Early MRDneg, achievement of MRD negativity within 6 weeks after chemotherapy initiation; Late MRDneg, achievement of MRD negativity more than 6 weeks after chemotherapy initiation; and MRDpos, persistent MRD detection during chemotherapy. The endpoint was disease-free survival (DFS), calculated from the date of achieving remission.

Results: The median age was 38 years (16–73), and the median follow-up time was 47 months (4–106). There were 8, 14, and 5 patients with early MRDneg, Late MRDneg, and MRDpos, respectively. There were no differences in patient characteristics by bone marrow status, except for the duration to achieving remission (Table 1). There were significant differences in the 3-year DFS rates among patients with early MRDneg, late MRDneg, and MRDpos (100% vs 72.9% vs 20%; p=0.001) (Figure 1). Patients undergoing transplantation had better prognosis than those receiving chemotherapy alone in the late MRDneg group (100% vs 40%; p=0.028), whereas there was no difference in the early MRDneg group (100% vs 100%; p=0.48).

Table 1. Patient characteristics by MRD status as assessed with the modified BIOMED-2 PCR for IgH protocol.

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<thead>
<tr>
<th>MRD neg</th>
<th>Late MRD neg</th>
<th>Late MRD pos</th>
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<tr>
<td>Early</td>
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<tr>
<td>3-year DFS</td>
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<td>100%</td>
<td>72.9%</td>
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Figure 1. The status of minimal residual disease was associated with prognosis.

Summary/Conclusions: The modified BIOMED-2 PCR protocol is a highly accurate and reliable method of MRD assessment in adult ALL. It predicted treatment outcomes in adult Ph- ALL, and patients with late MRDneg might derive a high survival benefit from allogeneic transplantation. Finally, the accuracy and reliability of the modified BIOMED-2 PCR for IgH were confirmed with a comparison to quantitative real-time PCR for BCR-ABL using samples from patients with Philadelphia-positive ALL (data not shown).

PB1631

SYSTEMATIC LITERATURE REVIEW OF PEGASPARGASE FOR THE TREATMENT OF NEWLY DIAGNOSED ADULTS WITH ACUTE LYMPHOCYTIC LEUKEMIA

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Background: Asparaginase is a component of a multi-agent chemotherapy regimen widely used in clinical practice for treatment with acute lymphocytic leukemia (ALL). Since 2006, pegaspargase (PEG-ASP) has been the gold standard asparaginase for the treatment of pediatric ALL as it offers equivalent efficacy to native E. coli L-asparaginase (native ASP), with less frequent dosing,
an IV administration option, and improved immunogenicity. Clinical outcomes in the adult ALL population are less well understood.

Aims: To assess the relative clinical benefit of PEG-ASP vs native ASP in 1st line treatment in newly diagnosed adult ALL patients in terms of event-free survival (EFS) and overall survival (OS). Safety outcomes were also examined.

Methods: A systematic literature search was conducted using a standardized search algorithm within the National Library of Medicine PubMed database to identify available evidence for newly diagnosed patients treated with adult ALL protocols that use PEG-ASP or native ASP. Randomized, observational, and cohort studies were included, with the predefined clinical outcomes of event-free-survival (EFS) and overall survival (OS). Data was pooled with 95% confidence intervals (CIs) calculated using the logit transformation.

Results: A total of 30 studies were identified that met the pre-specified inclusion criteria, with 10 studies providing data for PEG-ASP and 23 studies for native ASP. The pooled estimate of 2-year EFS for adult ALL patients treated in 1st line with asparaginase was 48.0% (95% CI: [10.8; 85.2]) for PEG-ASP and 66.0% (95% CI: [52.0; 77.9]) for native ASP. Similarly, the pooled estimate of 5-year OS was 64.5% (95% CI: [61.5; 67.5%]) for PEG-ASP and 46.8% (95% CI: [33.6; 60.1]) for native ASP. In very high risk ALL patients, the pooled estimate of 5-year OS was 57.1% (95% CI: [52.4; 61.7%]) for PEG-ASP and 35.3% (95% CI: [21.7; 51.7]) for native ASP.

Findings for safety outcomes were consistent with product labeling for both asparaginas.

Summary/Conclusions: The systematic literature review highlights a positive clinical effectiveness profile for PEG-ASP in regards to EFS and OS in the treatment of newly diagnosed adult ALL patients with less frequent administration and similar safety profile as compared with native ASP.

PB1632

A COMPREHENSIVE ANALYSIS OF PATIENT- AND THERAPY-RELATED FACTORS AFFECTING THE TOXICITY OF PEGYLATED-ASPARAGINASE FOR THE TREATMENT OF ADULT ACUTE LYMPHOBlastic LEUKEmIA

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Background: The application of pediatric regimens in the treatment of adult acute lymphoblastic leukemia (ALL) has led to a significant improvement in patients outcome. However, concerns about the feasibility of more intensive therapies and of the use of pegylated-L-Asparaginase (PEG-ASP) in adult patients have emerged. Some patient-related risk factors as high BMI or hepatic steatosis have been already identified as risk factors, but few data are available on the synergic toxic effect from other concomitant drugs.

Aims: The aim of this study was to evaluate the incidence of PEG-ASP related adverse events in a cohort of adult ALL patients in order to identify potential patient and therapy-related risk factors contributing to toxicity.

Methods: Since 2013, 21 adult ALL patients received PEG-ASP therapy in our institution. Median age was 44 (range 19-76); 12 patients were treated in front- line setting (7 according to a full pediatric protocol) whereas 9 patients received therapy for relapsed/refractory neoplasm. We retrospectively analyzed each single course which included PEG-ASP administration as an independent event, accounting 41 episodes. Patients’ features (age, BMI, disease status) and concomitant therapies were accurately analyzed as factors potentially affecting PEG-ASP toxicity. The incidence of major thrombotic/bleeding complications and grade III/IV hepatic or pancreatic toxicity was analyzed; toxicity grading and management of PEG-ASP related complications were performed according to guidelines recently published by Stock et al.

Results: No grade III/IV pancreatic, thrombotic or hemorrhagic adverse events were recorded. A total of 8 episodes of grade III/IV hepatic toxicities were observed. In 3 cases, grade IV toxicity was observed. Those patients experienced unexplained severe weight gain and painful epigastralgia, a common picture resembling sinusoidal occlusive disease, ultrasonography showed acute liver steatosis. All 3 patients received concomitant therapy with idarubicin, vincristine and vancomycin. In univariate analysis, the incidence of grade III/IV hepatic toxicity was significantly higher when concomitant chemotherapy with at least 2 mg/kg cumulative dose of vincristine (p = 0.044, HR 4.75) or at least 16 mg/kg cumulative dose of idarubicin (p = 0.046, HR 1.45) was administered. Steroids therapy determined a borderline increase in toxicity risk (p = 0.068, HR 1.5). No increase in toxicity was observed with any dosing of daunorubicin, cyclophosphamide, cytarabine, methotrexate and 6-mercaptopurine (Table 1). Among concomitant antibiotic therapies, vancomycin administration seemed to increase the incidence of grade III/IV hepato-toxicity (p = 0.02, HR 1.863). No significant increase was observed with carbapenem and azoles (Table 2). Receiving PEG-ASP as a first treatment, a high BMI (>25) were not related with an increased incidence of grade III/IV hepatic toxicity (Table 1). Notably, none of the patients undergoing full pediatric induction (who received the highest doses of PEG-ASP), regardless of age (ranging from 21 to 55) experienced grade III/IV hepatopathy. A multivariate logistic regression analysis disclosed that concomitant administration of idarubicin, vincristine or vancomycin were independent predictors of grade III/IV hepatotoxicity (p = 0.004, 0.027 and 0.042, respectively, Table 1).

Table 1.
**Summary/Conclusions:** The results of this study inform the magnitude of cost in Germany associated with adult rALL patients who or without an HSCT after relapse. The cost estimates provide a benchmark against which new treatment options for rALL can be compared. For future studies, it would be important to determine the magnitude of benefit such as long-term survival and other health consequences associated with HSCT as well.

**PB1634**

RETROSPECTIVE STUDY OF ADULT ALL IN MEXICO CITY: FIRST REPORT OF THE WORKING GROUP ON ACUTE LEUKEMIA

**Background:** The prognosis of adult acute lymphoblastic leukemia (ALL) is dire, with a long-term survival of 40-50%. This disease entity is probably more frequent in the Latino population. Several studies have reported a worse prognosis in Hispanics with ALL as well as a greater incidence of the Ph-like genetic signature; however, the data is inconclusive in the Mexican population and there are no existing large multicenter series of ALL patients in Mexico that analyze survival.

**Aims:** The aim of this study was to describe the incidence, clinical and biological characteristics as well as the survival of ALL patients in 5 referral hospitals in Mexico City.

**Methods:** A working group known as the Grupo de Trabajo de Leucemia Aguda (GTLA), was created as a result of an initiative of the Mexican Group for the Study of Hematology (Agrupación Mexicana para el Estudio de la Hematología) to promote acute leukemia research in Mexico. This is the first report of the GTLA which includes 5 referral hospitals in Mexico City. A retrospective, multicenter, descriptive study of adult ALL patients treated between 2009 and 2015 was conducted.

**Results:** We included 559 adults in 5 centers in Mexico City. Their median age was 28 years (14-81): adolescents and young adults (AYA) 67.3%; adults 24.7% and elderly adults 8.1%. Tumor lysis syndrome was detected in 9.8% of patients. Cytogenetic information was unavailable in 45% of cases due to lack of access or growth in metaphase. Among cases that could be analyzed, a normal karyotype was the most frequent (70.5%), followed by Ph+ (16.7%). Patients were considered high-risk in 52.1% cases. The most frequently used drug protocol was Hyper-CVAD, in 47% of cases. Complete remission (CR) was achieved in 67.1% of patients, and 18% required a second cycle for CR, while 13% were primarily refractory. A mortality rate during induction was registered as 10.6%, and there were 11.4% deaths while in CR. Among patients in CR, 59.1% relapsed. At the time of analysis, 26.7% of patients were alive, with a median OS of 12.97 months and a DFS of 16 months. Only 5.7% were able to receive an allogeneic hematopoietic progenitor cell transplant (AlloHCT). The median follow-up time in 36 patients was 22 months (range: 0.5-84 month). 4 deletions were determined (del 4-7, del 4-8, del 2-7, del 2-8)). The median OS for patients with BCR-ABL1- neg ALL with IKZF1 mutations and without OS was significantly better with first CR duration≥6 month and time to transplant≥2 months.

**Summary/Conclusions:** Allo-HSCT is an effective salvage treatment option for patients with refractory and relapsed ALL. Our retrospective analysis showed that R/R ALL with different status prior transplant had similar outcome post transplantation.

**PB1635**

THE FREQUENCY AND PROGNOSTIC SIGNIFICANCE OF IKZF1 DELETIONS IN ADULT PH-POSITIVE AND PH-NEGATIVE B-CELL ACUTE LYMPHOBластIC LEUKEMIA PATIENTS TREATED IN RUSSIAN ACUTE LYMPHOBLASTIC LEUKEMIA STUDIES

**Background:** The incidence of IKZF1 gene deletions is approximately 20% in adult patients with BCR-ABL1- negative B-cell ALL and 70–80% in BCR-ABL1- positive ALL. These mutations are associated with poor prognosis in patients with Ph-positive ALL, but not in patients with Ph-negative ALL, suggesting that Ph-negative deletions may be more prognostically valuable in patients with Ph-negative ALL.

**Aims:** To evaluate the frequency and prognostic impact of mutation status of IKZF1 in patients with de novo BCR-ABL1-negative and BCR-ABL1-positive B-cell acute lymphoblastic leukemia.

**Methods:** The study included 154 patients with Ph-negative ALL (median age 27, range 17-76; m:f=15:21) with newly diagnosed BCR-ABL1- neg B-cell ALL and 15 patients (median age 34 years, range 22-68; m:f=6:9) with BCR-ABL1- pos B-cell ALL, who were enrolled in Russian acute lymphoblastic leukaemia (RALL) - 2009 [ClinicalTrials.gov public site; NCT01193933] and RALL-2012 protocols since Feb 2010 till Sep 2016 and Aug 2009 till Feb 2017, respectively. Intragenic deletions of IKZF1 were detected using breakpoint-specific fluorescent multiplex polymerase chain reaction according to the procedure described by [Aurelie Caye et al, Haematologica, 2013]. DNA for PCR was extracted from leukaemia cells of frozen bone marrow samples.

**Results:** The IKZF1 deletions were detected in 7 (47%) of 15 patients with BCR-ABL1- pos ALL (3 cases with del 4-7 (43%), 2 - del 2-7 (28%), 1 – del 2a-8 and 1 – del 4-8 (14%)). The median follow-up time in 15 patients was 18 months (range: 4-79 month). Five patients died (33%) after relapse or progression of the disease, and 10 patients are alive. Overall survival (OS) for BCR-ABL1- pos B-cell ALL patients with IKZF1 mutations and without was 37.5% and 57.1% (p=0.77), relapse - free survival (RFS) - 25% and 33.3% (p=0.88), respectively. In patients with BCR-ABL1- neg ALL the IKZF1 deletions were revealed in 8 (22%) of 36 patients (4 cases with del 4-7 (50%), 2 - del 2-7 (25%), 1 – del 2a-8 (12.5%) and 1 in patient all types of deletions were determined (del 2-7, del 4-7, del 2a-8, del 4-7)). The median follow-up time in 36 patients was 22 months (range: 0.5-84 month). 4 patients died of the disease (11%) and 2 of infections. 30 patients are alive. OS for patients with BCR-ABL1- neg ALL with IKZF1 mutations and without was 100% and 60.2% (p=0.77), RFS - 75% and 40.2% (p=0.74), respectively. IKZF1 mutations seemed to be of poor prognosis for BCR-ABL1- neg ALL patients compared to the patients with Ph-negative ALL.

**Summary/Conclusions:** The frequency of IKZF1 gene deletions in patients with BCR-ABL1- pos and with BCR-ABL1- neg ALL was 47% and 22%, respectively. IKZF1 mutations seemed to be of poor prognosis for BCR-ABL1-
PB1637
GMALL BASED PROTOCOL, USING NATIVE E. COLI L-ASPARAGINASE, IMPROVES SURVIVAL OF ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA IN BRAZIL
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Background: Despite being the most common childhood cancer, nearly one half of ALL cases occurs in adults. Recently, it has been suggested that more intensive protocols may improve survival in adolescents and young adults (AYA).

Aims: Compare results of patients treated with BFM-based protocol to those patients treated with GMALL-based protocol, in a developing country.

Methods: This is a single center retrospective study which included all newly diagnosed adult ALL patients admitted between May/2012 and October/2016. Initially, patients aged 18-39 years (AYA group) were treated with BFM ALL 2009-based protocol and those aged 40-59 years were treated with GMALL 2003-based protocol. Since September 2013, because of high toxicity, only patients under 30 years were eligible for BFM-based treatment. Major adaptations were: (1) native E. coli l-asparaginase was substituted for peg-asparaginase, and (2) GMALL irradiation therapy was postponed to maintenance phase.

BFM/ABL1 positive patients received standard chemotherapy plus imatinib. Negative MRD was defined as <0.01% by flow cytometry. Overall survival was estimated by Kaplan-Meier method. Competing risk analysis was carried out for cumulative incidence of death in CR1 or not in CR1. This study was approved by local Ethics Committee.

Results: Thirty five patients were included, 21 of them started BFM-based treatment and 14 started GMALL-based protocol. During the first three months, 7 patients migrated from BFM to GMALL-based treatment because of toxicity and were analyzed separately. Median age was 21 years (18-38) for BFM group, 44 years (30-57) for GMALL-based, and 33 years (21-38) for de-escalated. Male predominance was observed (71%), not different between groups.

T-phenotype was more frequent than expected, representing 50% of BFM-based, 50% of GMALL-based and 29% of de-escalated groups. BCR/ABL1 was detected in 14% of BFM-based, 23% of GMALL-based and 14% of de-escalated groups (p=0.85). Seven patients (2 BFM and 5 GMALL) underwent allogeneic stem cell transplantation in first remission. Of all 35 patients, 31 achieved complete remission after first induction phase. With median follow-up of 18 months, 1-year overall survival (OS) was 60% for all patients (39% for BFM-based, 75% for GMALL-based and 86% for de-escalated groups – p=0.04; BFM-based versus other protocols). Cumulative incidence (CI) of death in first complete remission (CR1) at 12 months was 18%, not different between groups. CI of death at 12 months in non-CR1 (relapsed or refractory) patients was 39% for BFM-based, 7% for GMALL-based and 0% for de-escalated groups – BFM-based versus other CR 2.6; p = 0.13. Among 31 patients who achieved CR1, MRD data was available for 26 (74%) of these at the end of first induction. OS at 18 months for CR1 patients with negative MRD after first induction was 74%, compared to 52% in MDR+ (Figure 1).

Summary/Conclusions: Our results show that GMALL-based protocol yields good overall survival in adults ALL patients in a low income country, despite major adaptations. On the other hand, overall survival of AYA patients treated with BFM-based protocol was surprisingly poor, specially because of ineffective disease control. This finding may be related to several aspects: socioeconomic status, inadequate supportive care for more intensive therapies and ineffective cancer care network. Future prospective studies should focus on this issues.

PB1638
THE INVESTIGATION OF RELATIONSHIP BETWEEN COL1A1 AND FOK1 GENE POLYMORPHISMS AND DEVELOPMENT OF TREATMENT-RELATED SKELETAL COMPLICATIONS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA
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Background: Cure rates for childhood acute lymphoblastic leukemia (ALL) have approached 90% with therapeutic advances over the last several decades. Many treatment related long-term complications including impaired physical growth, neurocognitive dysfunction, emotional and occupational difficulties, cardiac abnormalities, hypertension, secondary neoplasms, decreased bone mineral density (BMD) and osteonecrosis have been observed as the number of survivors increased. Bone infiltration of leukemic cells, corti- costeroioid exposure, poor nutrition, low vitamin D levels, poor muscle mass, genetic predispositions contribute to the development or worsening of bone pathology during therapy that may result in osteoporosis, fracture and osteonecrosis.

Aims: In this study, we aimed to investigate whether vitamin D receptor and collagen protein gene polymorphisms, which are important in bone mineral and matrix formation, have effects on bone turnover in patients with ALL.

Methods: Fifty children with ALL who were diagnosed and treated with BFM-95 protocol (25 girls, 25 boys) between 1998-2008 and 96 healthy children at Dokuz Eylül University Medical School were enrolled in this study. Polymorphisms of vitamin D receptor (VDR) Fok1 gene and the collagen Col1A1 gene were studied from peripheral blood samples of the patients that were collected before initiation of chemotherapy protocol. After genomic DNA extraction, VDR Fok1 gene and colloidal Col1A1 gene polymorphisms were analyzed by poly- merase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). The data including age, sex, leukemia risk group, presence or absence of relapse were all noted. Bone marrow density and markers of bone metabolism including serum calcium, phosphorus, serum alkaline phosphatase, parathyroid hormone and 25-OH D vitamin levels were all screened before initiation of maintenance treatment.

Results: The distribution of Fok1 and Col1A1 gene polymorphisms was similar both in the patient group and healthy control group. The frequency of gene polymorphisms in the patient group were 8% FF, 46%FF and 46%FF for the Fok1 genotype and 62%GG, 26%GT and 12%TT for the Col1A1 genotype. Out of 50 patients, 16 (32%) patients were found to have skeletal diseases like osteopenia (16%), osteoporosis (12%) and osteonecrosis (8%). The Fok1 genotype and Col1A1 genotype polymorphisms were similar in both group of patients with or without skeletal diseases. The frequency of osteopenia was significantly higher in the male group (p=0.049) and the frequency of osteonecrosis was significantly higher in patients older than 10 years old (p=0.001). There was no significant association between Fok1 and Col1A1 gene polymorphisms and leukemia subtype, risk group or relapse rate.

Summary/Conclusions: It has recently become more important to prevent treatment-related complications that we see as a consequence of high cure rates in ALL. In this context we have investigated whether there is a relationship between gene polymorphisms and treatment related skeletal diseases like...
PB1639
OUTCOME OF ADOLESCENTS AND YOUNG ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA TREATED WITH PEDIATRIC PROTOCOL: MONOCENTRIC STUDY

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Background: Several retrospective studies have confirmed that adolescents and young adults (AYA) with acute lymphoblastic leukemia (ALL) treated with pediatric protocols have better outcomes than similarly aged patients treated with adult protocols.

Aims: We reported results and feasibility of a pediatric-based protocol (EORTC 58951) in adolescents and young adults.

Methods: From January 2000 to December 2015, 72 patients aged 16 to 30 years with newly diagnosed ALL were treated, in the department of clinical hematology of Hedi Chaker Hospital, according to the pediatric protocol EORTC 58951. Further leukemia characteristics (Sex, White Blood cell count, Blasts phenotype, Cytogenetic results), we studied the protocol results: response to induction, risk group stratification (average: AR1 and AR2, very high: VHR), remission rate, death rate, relapse rate and 5 years survivals (over all OS and event free EFS).

Results: Seventy two AYA ALL were treated with the pediatric protocol. The patients were 45 males and 27 females (SR=1.66). A WBC>100 G/ L was noted in 32%. T ALL phenotype was noted in 53% of cases. Twenty two patients (30%) were PPR. Nine patients (13%) were treated according AR1 arm, 39 patients (54%) according AR2 arm and 24 patients (33%) according VHR arm induction. CR rate was 87% after one course and 94% after 2 courses. Induction death was noted in 3% and post-induction death was noted in 13%. Twenty four patients were in AR1 or AR2 arm. Of the patients eligible for allogenic stem cell transplantation (SCT), among them 15 patients had a familial donor and 10 patients 2 at the High Risk group) experienced disturbances during the reinduction period of time ranging of 5 days to 6 months. One patient experienced a psychiatric event during induction (Prot.II,phase 2) with aggression and violence towards others and had to be treated immediately with intramuscularly haloperidol and diazepam. All of our patients are alive and in remission, 7 off therapy for a period of 3 years, and 2 receiving maintenance therapy. Statistical analysis showed that severe psychiatric disturbances were observed more frequently in older patients and they were more common with the administration of dexamethasone than with prednisolone.

Summary/Conclusions: Severe psychiatric disturbances are not infrequent in adult and adolescent patients receiving treatment for ALL. Awareness and recognition of this complication, appropriate parental education for identifying early signs, and prompt therapeutic interventions are essential for optimal outcome. Further studies are required for identifying patients at risk and best use of chemotherapeutic agents and of dexamethasone.

PB1640
ASSESSMENT OF DEPRESSION AND SELF-CONCEPTION IN CHILDREN WITH ALL-TREATMENT

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Background: Leukemia is the most prevalent pediatric malignancy with acute lymphoblastic leukemia (ALL) being the most common accounting for 75% of leukemia cases with about 2400 newly diagnosed children each year worldwide. Treatment of ALL requires long course chemotherapy ranging up to 40 months with 20% possibility of relapse. Affected children receive inpatient treatment at the clinic for nearly six months for leukemia and related complications. The majority of the patients of the High Risk group and AR1 and AR2, very high: VHR, remission rate, death rate, relapse rate and 5 years survivals (over all OS and event free EFS).

Aims: The study aimed to investigate the incidence of severe psychiatric disturbances in patients treated for childhood ALL.

Methods: We report the results of a retrospective analysis of the incidence of severe psychiatric disturbances, defined as behavioral and psychological changes which lead to dangerous or erratic behaviors requiring use of psychotropic medications, in patients treated for childhood ALL. All patients were treated in a single institution and followed the same chemotherapeutic protocol, according to which, corticosteroids are administered initially during the “induction” phase and then in multiple subsequent pulses.

Results: Seventy patients (mean age:4.04 years old, range:1-16) were treated in this protocol. The incidence of psychiatric disturbances was noted in 12 patients (12.8%) children (6 boys, 3 girls) of mean age 12.3 years old (range: 10-15) experienced psychiatric - neurological symptoms and/or mental disorders, which included major depressive disorder, withdrawal, first psychotic episode, disorientation, visual hallucinations, mood swings and behavioral outbursts. The median age of the group at diagnosis was 3 years with newly diagnosed ALL were treated, in the department of clinical hematology of Hedi Chaker Hospital, according to the pediatric protocol EORTC 58951. Further leukemia characteristics (Sex, White Blood cell count, Blasts phenotype, Cytogenetic results), we studied the protocol results: response to induction, risk group stratification (average: AR1 and AR2, very high: VHR), remission rate, death rate, relapse rate and 5 years survivals (overall OS and event free EFS).

Results: Seventy two AYA ALL were treated with the pediatric protocol. The patients were 45 males and 27 females (SR=1.66). A WBC>100 G/ L was noted in 32%. T ALL phenotype was noted in 53% of cases. Twenty two patients (30%) were PPR. Nine patients (13%) were treated according AR1 arm, 39 patients (54%) according AR2 arm and 24 patients (33%) according VHR arm induction. CR rate was 87% after one course and 94% after 2 courses. Induction death was noted in 3% and post-induction death was noted in 13%. Twenty four patients were in AR1 or AR2 arm. Of the patients eligible for allogenic stem cell transplantation (SCT), among them 15 patients had a familial donor and 10 patients 2 at the High Risk group) experienced disturbances during the reinduction period of time ranging of 5 days to 6 months. One patient experienced a psychiatric episode during induction (Prot.II,phase 2) with aggression and violence towards others and had to be treated immediately with intramuscularly haloperidol and diazepam. All of our patients are alive and in remission, 7 off therapy for a period of 3 years, and 2 receiving maintenance therapy. Statistical analysis showed that severe psychiatric disturbances were observed more frequently in older patients and they were more common with the administration of dexamethasone than with prednisolone.

Summary/Conclusions: Severe psychiatric disturbances are not infrequent in adult and adolescent patients receiving treatment for ALL. Awareness and recognition of this complication, appropriate parental education for identifying early signs, and prompt therapeutic interventions are essential for optimal outcome. Further studies are required for identifying patients at risk and best use of chemotherapeutic agents and of dexamethasone.

PB1642
INCIDENCE, SEVERITY AND RISK FACTORS FOR NEUROLOGIC COMPLICATIONS ASSOCIATED WITH L-ASPARAGINASE TREATMENT IN PAEDIATRIC PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background: Combined chemotherapy increased over time cure rate in patients with acute lymphoblastic leukemia (ALL). Among other things, one of the direct adverse effects of chemotherapy is affecting hemostasis or bleeding. Hemostasis can also be affected indirectly by the appearance of infections secondary immunosuppression. It has been found that L Asparaginase induces thrombotic events by reducing antithrombin III, protein C, protein S and by inducing endothelial damage. Thrombotic events may result as a direct effect of the disease, may be the effect of chemotherapy or may be related with platelet abnormalities of the patient.

Aims: To evaluate incidence and severity of thrombotic or bleeding events in paediatric patients with ALL during chemotherapy.

Methods: We considered all patients hospitalized for ALL in the Pediatrics
Department of Clinical Institute Fundeni during 2010-2017 and received chemotherapy according to protocol ALL BFM 1995 and ALL BFM 2002, established after framing in the risk group.

**Results:** Over a period of 8 years in our department 280 patients with ALL received L-asparaginase in the induction phase. Neurological manifestation suggestive for bleeding or thrombotic events occurred in 9/280 (3.21%) patients. 2 patients were treated according to protocol ALL BFM 1995 and 7 patients were treated according to protocol ALL BFM 2002. M/F ratio was 4/5. Patients had diagnosis between 3 and 15 years (median age 9 years). All patients had thrombotic events after starting administration of L-asparaginase during induction. Most had clinical symptoms after the fourth dose of L-asparaginase. Clinical manifestations were accompanied by hypofibrinogenemia (<100 mg/dl) especially in patients who experienced bleeding. The patients who experienced thrombosis had decreased levels of antithrombin III, protein C and increased D dimer levels. The diagnosis of cerebral venous sinus thrombosis (CVST) is typically based on clinical suspicion and imaging confirmation. At 5 of these patients neuroimaging tests demonstrated CVST, and at one documented CVST after developing neurological symptoms; one of the patients suffered major complication (extended brain injury) and died. All patients with ALL and thrombotic events received low-molecular weight heparin (LMWH) for 3 to 6 months. A follow-up CT or MRI at 3 to 6 months after diagnosis was made to assess for recanalization of the occluded cortical veins/sinus. Survival in the patients with CVST was 84.6%. 1 patient with ALL and hemostasis alteration had intracerebral hemorrhage (ICH) with rapid progressive neurological deterioration to death. 1 patient had pulmonary embolism associated with clotting disorders and severe sepsis and he died. 2 patients had clinical manifestation (headache, confusion and seizures) and clotting disorders (decreased levels of antithrombin III, protein C, fibrinogen and increased D dimer levels), but with normal brain imaging. Survival in the cohort was 77.7%.

**Summary/Conclusions:** Thrombotic events have occurred in all patients during induction. Clinical manifestation were depending on size, and duration of thrombosis, from headaches, seizures or focal neurological deficits. Severe sepsis association was an additionally risk factor for thrombotic and bleeding events in patients with ALL. Screening for genetic prothrombotic defects diagnosis prior to initiating chemotherapy may represent a way to reduce thrombotic or bleeding events and appropriate management of hemostasis disorders that occur during the treatment.

**PB1643**

**INCIDENCE AND SURVIVAL OF CHILDHOOD LEUKAEMIA IN ARMENIA: A POPULATION-BASED ANALYSIS**

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**Background:** Leukaemia is the most common cancer in children. Childhood leukaemia incidence and survival varies globally, and this could be associated with risk factors, genetics, and improvement in diagnosis and treatment. Armenia is considered to be a mono ethnic nation.

**Aims:** To characterize long-term survival outcomes including leukemia-free survival (LFS) and overall survival (OS) for Ph+ ALL patients treated with imatinib versus dasatinib.

**Methods:** Retrospective chart review was conducted at our institution. Patients >= 18 years old and diagnosed with Ph+ ALL between 2002 and 2015 were included. Analysis was done by intent to treat for patients initiated with imatinib or dasatinib at the time of Ph+ diagnosis. The primary endpoints were 2-year LFS and OS and secondary endpoints were complete molecular response (CMR; BCR-ABL1/ABL1 ratio <0.01% by PCR) and major molecular response (MMR; BCR-ABL1/ABL1 ratio <0.1% by PCR).

**Results:** Among 46 patients with Ph+ ALL, 74% (n=34) were in imatinib group and 17% (n=8) in dasatinib group; 9% were treated with other or no TKI (1 ponatinib and 3 with no TKI). Thirty-eight percent (n=13) of patients in imatinib group and 13% (n=1) in dasatinib group switched to a different TKI due to adverse effects or failure to achieve remission. There was a trend towards increased 2-year LFS for patients on dasatinib (HR 0.40, 95% CI: 0.14-1.14, p=0.09) and no difference in 2-year mortality (HR 1.00 95%CI: 0.46-2.17, p=0.09). Molecular response data was available for 65% (n=28) of patients; 75% of imatinib group achieved CMR or MMR (65% CMR) compared to 76% of dasatinib group (63% CMR) (p=0.98) (Figure 1).

**Figure 1.**

**Summary/Conclusions:** In conclusion, dasatinib, compared to imatinib, in combination with chemotherapy, may prolong LFS in patients with Ph+ ALL and may be a suitable first-line agent. Large, randomized studies are needed to better define a detailed treatment protocol in this high-risk patient population.

**PB1645**

**OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN A MIXED COHORT OF PEDIATRIC AND ADULT PATIENTS WITH KMT2A-AFF1 ACUTE LYMPHBLASTIC LEUKEMIA**

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**Background:** Acute lymphoblastic leukemia (ALL) with positive Philadelphia chromosome (Ph+) is a unique subset of ALL with poor prognosis. Recent studies have demonstrated improved survival outcomes in adult patients with Ph+ ALL with the use of tyrosine kinase inhibitors (TKIs) along with chemotherapy. However, there are very few studies that describe the comparative effectiveness of various TKIs in this patient population.

**Aims:** To characterize long-term survival outcomes including leukemia-free survival (LFS) and overall survival (OS) for Ph+ adult ALL patients treated with imatinib versus dasatinib.

**Methods:** Retrospective chart review was conducted at our institution. Patients >= 18 years old and diagnosed with Ph+ ALL between 2002 and 2015 were included. Analysis was done by intent to treat for patients initiated with imatinib or dasatinib at the time of Ph+ diagnosis. The primary endpoints were 2-year LFS and OS and secondary endpoints were complete molecular response (CMR; BCR-ABL1/ABL1 ratio <0.01% by PCR) and major molecular response (MMR; BCR-ABL1/ABL1 ratio <0.1% by PCR).

**Results:** Among 46 patients with Ph+ ALL, 74% (n=34) were in imatinib group and 17% (n=8) in dasatinib group; 9% were treated with other or no TKI (1 ponatinib and 3 with no TKI). Thirty-eight percent (n=13) of patients in imatinib group and 13% (n=1) in dasatinib group switched to a different TKI due to adverse effects or failure to achieve remission. There was a trend towards increased 2-year LFS for patients on dasatinib (HR 0.40, 95% CI: 0.14-1.14, p=0.09) and no difference in 2-year mortality (HR 1.00 95%CI: 0.46-2.17, p=0.09). Molecular response data was available for 65% (n=28) of patients; 75% of imatinib group achieved CMR or MMR (65% CMR) compared to 76% of dasatinib group (63% CMR) (p=0.98) (Figure 1).

**Figure 1.**
Results: Eight of 21 (38%) patients exhibited an isolated t(4;11) translocation, Additional chromosome abnormalities (ACAs) were revealed in 11 (52%) patients, including 8 (42%) subjects with 3 and more chromosome aberrations. In univariate analysis, significance was shown for clinical stage at HSCT (1st remission vs other stages, 75% vs 0%; p=0.001 for OS; 58% vs 0%, p<0.001 for EFS), complex chromosomal aberrations (<3 abnormalities vs ≥3 abnormalities, p=0.04 for OS; 46% vs 0%, p=0.04 for EFS). According to multivariate analysis, the clinical stage at HSCT (HR 26.8, 95% CI 3.28-218.80; p=0.002 for OS; HR 11.18, 95% CI 2.92-42.80 p=0.0004 for EFS) was only independent prognostic factor for clinical outcome.

Summary/Conclusions: The study has shown the stage of disease at the moment of allo-HSCT to be independent prognostic factor in a mixed cohort of KMT2A-AFF1 ALL patients treated with HSCT. The good results of allo-HSCT can be obtained using a haploidentical transplantation from parents that removes the problem of searching the HLA-matched donors in the Registers and, therefore, greatly simplifies the treatment.

PB1646
Dermatological complications associated with tyrosine kinase inhibitors for the treatment of acute leukemia
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Background: Despite of targeted effects of tyrosine kinase inhibitors (TKIs), they are not absolutely selective in relation to their target. Hair pigmentation is regulated by factors including the interaction of the ligand stem cell factor (SCF) with its class III receptor tyrosine kinase, c-kit. Hair depigmentation observed during therapy TKI with action directed against class III receptor tyrosine kinase (PDGFRA, PDGFRB, C-KIT, CSF1R, FLTR3). But other TKI such as BCR/Ab1 TKI can also inhibit class III receptor tyrosine kinase by non-targeted actions. Skin reactions are the most common observed during the epidermal growth factor receptor-tyrosine kinase inhibitor treatment.

Aims: To describe the spectrum of skin and hair reactions in patients with acute leukemias (Ph+/Ph- acute lymphoblastic leukemia and acute myeloid leukemia) during the treatment by second-generation TKI (bosutinib), third-generation TKI (ponatinib) and multikinase inhibitor (sorafenib).

Methods: From 2016 to March 2017 6 patients (pts), age 24-53 (median 29,5), 1 male, 5 female, received second or third line therapy with target tyrosine kinase inhibitors in National Research Center for Hematology. One pt (pt 1) with AML had been receiving chemotherapy (decitabine, cytarabine, idarubicin) with continuous treatment of sorafenib. Three pts with Ph+ ALL received TKIs. Two of them with T315I mutation (pts 2, 3) received ponatinib and one of ph+ ALL, without molecular remission on dasatinib and nilotinib therapy, received second-generation TKI (bosutinib). One pt with B-ALL was treated by sorafenib due to refractory disease on the first-line therapy (pt 5). And one patient (pt 6) with T-cell ALL received sorafenib with nelarabine containing chemotherapy due to early relapse after allogeneic stem cell transplantation.

Figure 1.

Results: All of the 6 patients who had taken second-generation TKI (bosutinib), third-generation TKI (ponatinib) and multikinase inhibitor sorafenib developed dermatologic reactions (skin rash or grey hair). Generalized maculo-papular skin rash grade II evolved after 2 weeks of sorafenib treatment in pt1. Both patients on ponatinib therapy developed localized maculo-papular skin rash grade I in pt 2 after 8 weeks of therapy. In pt 3 after 6 weeks of ponatinib treatment gray hair observed. Skin rash with pigmentation grade I evolved in pt 3 after 12 weeks of therapy. Pt 4 had gray hair after 12 weeks second-generation TKI (bosutinib) treatment. Palmar-plantar erythrodysesthesia syndrome grade II and hair and total skin depigmentation were evolved after 2 weeks and after 4,5 months, respectively observed during the sorafenib treatment. Pt 5 (with psoriasis anamnesis) Pt 6 developed localized maculo-papular skin rash grade I after 5 weeks of sorafenib treatment. Despite of all patients developed dermatological side effects, temporarily discontinuation of TKI therapy was required in only three (50%) cases. In the other cases the treatment was continued. The therapy was restarted in all pts with temporarily discontinuation after skin lesions disappearing (Figure 1).

Summary/Conclusions: Dermatological adverse events in acute leukemia pts who have taken second-generation TKI (bosutinib), third-generation TKI (ponatinib) and multikinase inhibitor sorafenib they were not serious. Temporarily dose reduction or interruption of TKI therapy led to disappearance of skin lesions. Restarting TKI at full dose did not lead to dermatological adverse reactions reappearing. Moreover, the temporary cancellation did not reduce its effectiveness.
SEVERE HYPOFIBRINOGENEMIA ASSOCIATED WITH IMATINIB AND PREDNISONE THERAPY IN PHILADELPHIA CHROMOSOME–POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Hypofibrinogenemia associated to acute lymphoblastic leukemia (ALL) is rare and usually due to L-asparaginase. Consumption coagulopathy or therapy-related hematotoxicity are other possible explanations. Severe hypofibrinogenemia, not linked to the causes listed, was rarely reported and a role of steroid therapy on fibrinogen metabolism was suggested.

Aims: Our aim was to identify the incidence of severe hypofibrinogenemia during induction phase in a cohort of consecutive ALL patients and to assess its impact on clinical decision-making.

Methods: In order to avoid confounding factor due to L-asparaginase, we revised our cohort of Philadelphia chromosome–positive (Ph+) ALL that we treated according to pediatric-type therapy program (imatinib, intensive chemotherapy without L-asparaginase) for patients aged 18-65 years and through LAL0201-B protocol (imatinib, prednisone) for patients ≥65 years. We retrospectively analyzed coagulation tests on admission and during induction therapy of all Ph+ALL patients diagnosed at our Institution from 2004.

Results: Twenty-one Ph+ALL were identified: 17 patients were younger than 65 years, while the remaining 4 patients had a median age of 74 years (66-76). No alteration of plasma fibrinogen during induction was observed in younger patients. Severe hypofibrinogenemia (≤100 mg/dl) was detected in 3 out of 4 Ph+ALL over 65 years. In these patients induction consisted of prednisone 40 mg/m² for 6 days, followed by 1 to 45 and imatinib at the fixed dose of 800 mg/d. On admission hemoglobin levels were ≥10 g/dl in all patients, leucocytes counts were 2×10⁹/L (blasts 15%), 8×10⁹/L (blasts 30%) and 18×10⁹/L (blasts 61%), while platelet count was reduced in 2 cases (61x10⁹/L and 65x10⁹/L). Coagulation tests remained in a normal range; platelet counts showed a trend to normalization. Early clearance of peripheral blood blasts was observed and when hypofibrinogenemia appeared no blast cells were detectable. At the end of induction bone-marrow evaluation demonstrated the absence of BCR-ABL transcript by qualitative RT-PCR. There were no bleeding events and only one patient received a prophylactic transfusion of fresh-frozen plasma (10 ml/kg) for fibrinogen <50 mg/dl on two occasions. Normal fibrinogen levels (≥165 mg/dl) were recovered at the end of steroid therapy.

Summary/Conclusions: We observed severe hypofibrinogenemia in Ph+ALL patients older than 65 years treated with imatinib and high-doses steroid, while normal fibrinogen levels were detected in younger Ph+ALL during intensive chemotherapy plus imatinib. In our experience, hypofibrinogenemia was not associated to major bleeding events, although its clinical significance should be investigated in larger series. Fibrinogen may recognize multiple metabolic pathways, also unrelated to in vivo coagulation and fibrinolysis; the correspondence between steroid treatment and hypofibrinogenemia seems to suggest that glucocorticoids may alter some steps in fibrinogen kinetics and could be considered as a cause of acquired hypofibrinogenemia.

LATE EFFECTS OF CHEMORADIOThERAPY ON THE ENDOCRINE SYSTEM IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Over the past four decades treatment of childhood acute lymphoblastic leukemia has been modified with the aim of achieving high survival rate while reducing the risk of the life threatening late-effects and promoting risk-based follow-up care of survivors.

Aims: The aim of our study is evaluation of late effects of chemotherapy and cranial radiotherapy on the endocrine system in children with acute lymphoblastic leukemia.

Methods: Forty-eight patients, who were diagnosed and treated for ALL between 1997-2007 in Istanbul Kanuni Sultan Suleyman Education and Research Hospital Pediatric Hematology-Oncology Clinic and have disease-free for at least 5 years after cessation of treatment, were evaluated prospectively. The study form included each patients age, gender, weight, height, target height, parental height, treatment protocol, stage of puberty, bone age, TSH, free T4, LH, FSH, estradiol or testosterone, IGF-1 and IGFBP-3 levels. Annual rate of growth was evaluated for each patient. The patients with inadequate growth rate and delayed bone age were subjected to growth hormon stimulation test with clonidine.

Results: Mean age of the patients was 14.4±2.85 (10.5-22.4) years. Thirty-one of patients had prophylactic cranial radiotherapy; five of them 18 Gy and twenty-six had 12 Gy CRT. Fifteen of the 48 patients were diagnosed with at least one endocrinological disorder. Six patients had lower height (<-2 SD), three patients had a body mass index >30kg/m². Bone age delayed in two patients. Four patients had IGF-1 value below <-2SD and two patients had inadequate levels of growth hormone. Tanner stage of the patients were appropriate for their ages except for one patient with hypergonadotropic hipogonadism and one patient with pubertas precox. Subclinical hypothyroidism was detected in two patients.

Summary/Conclusions: Significant late effects may develop over time in children treated for ALL. For this reason long-term follow-up of these children is necessary. Because of the awareness of the late effects the treatment modified to reduce the risk of the life threatening late-effects.
Acute myeloid leukemia - Biology

PB1650

MUTATIONAL ANALYSIS OF 231 DE NOVO AML PATIENTS BELOW 60 YEARS WITH CURATIVE THERAPY

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Background: Acute myeloid leukemia (AML) is an aggressive cancer disease of the myeloid lineage of blood cells, characterized by rapid growth of undifferentiated myeloid precursors. Analysis of the spectrum of somatic mutations in leukemic cells may help to improve the identification of individual prognostic subgroups of patients as well as to observe clonal evolution in the course of AML treatment.

Aims: The aim of the project is to identify somatic alterations in genes related to AML using next generation sequencing (NGS) in large cohort of AML patients from Czech Republic and to determine their frequency and mutual coexistence.

Methods: The analyzed group consists of 231 de novo consecutively diagnosed AML patients with curative therapy below 60 years from five hematological centers. The NGS libraries are prepared from peripheral blood samples from diagnosis using ClearSeq AML panel (Agilent Technologies) and sequenced on MiSeq and NextSeq machines (Illumina). Resulting variants are screened using in-house bioinformatic tools.

Results: At least one somatic mutation (median 2; range 0-6) was identified in 204 (88.3%) patients with de novo AML. In total, 526 recurrent mutations in 19 genes were identified. The most frequently mutated genes were: FLT3 91/231 (39.4%); from this FL37-TID 69/231 (29.9%) and FLT3-TKD 22/231 (9.5%). NPM1 90/231 (39.0%; mutation type A 71/90 [78.9%], type B 11/90 [11.1%], other types 10/90 [10.0%]), DNMT3A 68/231 (29.4%; mutations in codon R882 49/68 [72.1%]), NRAS 51/231 (22.0%; the most frequent mutation G12D 17/51 [22.0%]; 1151 patients [21.6%] contain more than one mutation in NRAS gene), IDH2 35/231 (15.2%) and CEPBA 35/231 (15.2%). The analysis also identified mutations in rarely mutated genes U2AF1, SF3B1, EZH2 and SETBP1 in 4/231 (1.7%), 4/231 (1.7%), 1/231 (0.4%) and 1/231 (0.4%) samples, respectively (Figure 1).

Summary/Conclusions: The results of mutational analysis of large cohort of AML patients show high heterogeneity of detected mutations. Surprisingly we have detected high percentage of patients with mutations in gene NRAS. Together with sequencing results from the time of remission/relapse/resistance of the disease, the data will enable to get more complex view on the development of AML in time.

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PB1651

INHIBITION OF LIN28B IMPAIRS LEUKEMIA CELL GROWTH AND METABOLISM IN ACUTE MYELOID LEUKEMIA

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Background: Current conventional chemotherapy for acute myeloid leukemia (AML) can achieve remission in over 70% of patients, but a majority of them will relapse within 5 years despite continued treatment. 2. The relapse is postulated to be due to leukemia stem cells (LSCs), which is different from normal hematopoietic stem cells (HSCs). LIN28B is microRNA regulator and stem cell reprogramming factor. 3. Overexpression of LIN28B has been associated with advanced human malignancies and cancer stem cells (CSCs), including AML. However, the molecular mechanism by which LIN28B contributes to the development of AML remains largely elusive.

Aims: 1. To study the function role of LIN28B in cell proliferation, cell cycle and colony formation ability of AML cells. 2. To systematically dissect transcriptional signaling mediated by LIN28B on whole genome level. 3. To determine the key targets of LIN28B in AML. 4. To explore the function of LIN28B in AML in vivo.

Methods: 1. We modulated LIN28B expression in AML and non-leukemic cells and investigated functional consequences in cell proliferation, cell cycle and colony forming assays. 2. We performed a microarray-based analysis for LIN28B expression in leukemic cells and interrogated gene expression data with different bioinformatic tools. 3. AML mouse xenograft model was used to examine the in vivo function of LIN28B.

Results: We first showed that increased LIN28B expression was associated with worse survival in AML patients. We demonstrated that targeting LIN28B in AML cells resulted in cell cycle arrest, inhibition of cell proliferation and colony formation, which was induced by de-repression of let-7a miRNA. On the other hand, overexpression of LIN28B promoted cell proliferation. Mechanistic studies revealed that inhibition of LIN28B induces metabolic changes in AML cells.

Conclusion: These findings uncover a novel mechanism of an important regulatory signaling, LIN28B/let-7a/GFP2B1, in leukemogenesis and provide a rationale to target this pathway as effective therapeutic strategy.

PB1652

Abstract withdrawn.

PB1653

EVALUATION OF MINIMAL RESIDUAL DISEASE IN NPM1-MUTATED AML PATIENTS

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Background: Minimal residual disease (MRD) tests provide early identification of hematologic relapse and timely management of AML patients. About 60% of adult normal karyotype AML has a mutation in exon 12 of NPM1 gene. This mutation is specific for malignant clone and potentially is a good marker of MRD.

Aims: The aim of the study was to analyze the usefulness of NPM1 as a marker for MRD quantification in AML during follow-up.

Methods: Retrospective study included 34 patients with mutated-NPM1 and treated with intensive chemotherapy (2009-2015). Bone marrow (188) and peripheral blood (277) samples were analyzed from complete remission (MRD NPM1 negative). NPM1 detection was performed by quantitative RT-PCR (Gorello et al. Leukemia 2006). Patients were considered positive when presented >1 NPM1 sample positive or/and one sample NPM1 >0.02%. Cox regression was used for univariate analysis.

Results: Patients were segregated in 2 groups: Relapse patients (Group 1: 32.2%, 11/34) and no relapse patients (Group 2: 67.6%, 23/34). Group 1 presented MRD NPM1 positive in 9/11 (82%) of patients, the time from NPM1 to relapse was 4.6 months (1.6-24). NPM1 mean was 1.7 (0.03-9). Group 2 presented MRD NPM1 negative (<0.02% y/ or 1 determination) in 21/23 (91%) patients. Univariate analysis was performed and our results show that age, leukocyte, LDH and MRD NPM1 are prognostic factors for cumulative incidence of relapse (Figure 1).

Figure 1.
Summary/Conclusions: NPM1 is a useful marker for MRD quantification in AML patients undergoing intensive therapy. NPM1 positive during follow-up is associated with a higher probability of relapse.

PB1654

AT101 ELIMINATES AML STEM CELLS VIA ACTIVATION OF INTRINSIC APOPTOTIC PATHWAY AND PARTICIPATION IN DNA DAMAGE RESPONSE

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Background: Leukemia stem cells (LSCs) are considered as the main reason for treatment failure and relapse in acute myeloid leukemia. Overexpression of Bcl-2 anti-apoptotic proteins is associated with the survival and self-renewal of LSCs.

Aims: To observe the effect for AT101 to eliminate AML stem cells and its underlying mechanism.

Methods: Use CD34+CD38-CD123+KG1α and primary AML CD34+ cells as research object.

Results: In this study, we demonstrated that AT101, a B3 mimetic pan-Bcl-2 inhibitor, was significantly and effectively cytotoxic towards CD34+CD38-CD123+KG1α and primary AML CD34+ cells, with slight effect on CD34+ normal hematopoietic cells. And the mechanism was closely associated with activation of intrinsic apoptotic pathway, such as loss of mitochondrial membrane potential and caspase activation, along with disturbance of DNA damage response. Further analysis on AML patients’ clinical characteristics revealed that the ex vivo efficacy of AT101 in primary samples was significantly correlated to hyperleukocytosis or FLT3-ITD mutation. Besides, AT101 exhibited exciting effect on CD34+ blasts from patients who are old or cannot achieve CR after induction therapy.

Summary/Conclusions: In conclusion, together, these findings provides potentially for the use of AT101 to treat relapse and refractory AML as alternative salvage regime in the future, including those clinically characterized by one or more adverse prognostic abnormalities.

PB1655

COOPERATIVE EFFECT OF CHIDAMIDE AND CHEMOTHERAPEUTIC DRUGS INDUCE APOPTOSIS BY DNA DAMAGE ACCUMULATION AND REPAIR DEFECTS IN ACUTE MYELOID LEUKEMIA STEM AND PROGENITOR CELLS

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Background: Lots of conventional chemotherapeutic drugs are confirmed to take effect to eliminate leukemia stem cells (LSCs) on account of higher DNA repair capacity of cancer stem cells than bulk cancer cells, which become the root of resistance and recurrence. Thus, new strategy to eliminate LSCs in AML is urgently needed.

Aims: To observe the effect of low dose chidamide in combination with chemotherapeutic agents on eliminating AML stem cells.

Methods: We used a novel benzamide-type HDAC inhibitors, chidamide in combination with DNA-damaging agents (daunorubicin, idarubicin and cytarabine) to treat CD34+CD38-KG1α cells and primary refractory or relapsed AML CD34+ cells.

Results: Here, we report that low dose chidamide, a novel benzamide-type HDAC inhibitors, which selectively targeted HDAC 1, 2, 3, 10, could enhances cytotoxicity of DNA-damaging agents (daunorubicin, idarubicin and cytarabine) in CD34+CD38- KG1α cells and primary refractory or relapsed AML CD34+ cells, reflected by inhibition of cell proliferation and induction of apoptosis in vitro. Mechanistically, these events were associated with DNA damage accumulation and repair defects. Co-treatment with chidamide and DNA-damaging agents IDA gave rise to production of yH2A.X, and cytarabine) in CD34+CD38- KG1α cells and primary refractory or relapsed AML CD34+ cells, reflected by inhibition of cell proliferation and enhancement of cytotoxicity of DNA-damaging agents (daunorubicin, idarubicin and cytarabine) to treat CD34+CD38- KG1α cells and primary refractory or relapsed AML.

Summary/Conclusions: These findings provide preclinical evidence for low dose chidamide in combination with chemotherapeutic agents to treat recurrent/resistant AML as an alternative salvage regimen, especially those possessed stem and progenitor cells.

PB1656

NEW CANDIDATE GENES USEFUL TO PREDICT THE RISK OF RELAPSE IN ACUTE PROMYELOCYTIC LEUKEMIA (APL)

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Background: Nowadays, Acute Promyelocytic Leukemia (APL) is a disease entity with a very high rate of cure and an estimated 2-year overall survival of 97%. Early death, rather than resistant disease so common in all other subtypes of AML, has emerged as the major cause of treatment failure, and relapse is a very rare occurrence.

Aims: To observe the effect of APL as a rare entity, and it is announced to become rarer with the advances in first line therapy. Molecular characteristics are hard to analyze without an effort to collect and bank samples together from multiple institutions. Since relapses, especially relapses out of follow-up period, represent a sudden life-treating condition for patients, to predict patients at higher risk of relapse we selected two candidate genes that could be involved in pathways favoring relapse.

Methods: We collected data of all the APL referred to our institution from 2014. Within 23 patients, we encountered 20 new diagnosis and 2 relapse of APL.

Results: We selected blasts in samples obtained from Bone Marrow with Single Nucleotide Polymorphisms Array Cytoscan HD.

Summary/Conclusions: By the analysis of ROBO1-2 and GRIP1 at the diagnosis stage of APL we could establish a different and strict follow-up program for patients with these alterations.

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AN INVESTIGATION INTO THE ROLE OF S100A8 AND S100A9 IN ACUTE MYELOID LEUKAEMIA
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Background: Acute myeloid leukemia (AML) is the a hematological malignancy characterised by the over proliferation and block in differentiation of clonally expanded leukaemia potential bone marrow cells such as S100A8 could assess the progression and remission of AML.

Aims: S100A8 and S100A9 (Ca2+ binding helix E-loop-helix-F hand), are inflammatory markers which are also suggested to promote chemoresistance by stimulation of autophagy. Microarray data from the Chevsutt lab shows that both S100A8 and S100A9 transcripts are downregulated by the BET-bromodomain inhibitor JQ1 in AML cell lines. We aimed to investigate this response in AML patient bone marrow samples and cell lines.

Methods: We used AML cell line including OCI-AML2, OCI-AML3 and THP-1 in addition to AML patient bone marrow samples and healthy volunteer samples. We carried out RT-qPCR and immunocytochemistry and western blotting techniques to look at levels of S100A8 and S100A9 in samples.

Results: Here we show that levels of S100A8 and S100A9 miRNA levels are suppressed in response to JQ1 in the AML cells lines OCI-AML2, OCI-AML3 and THP-1. We find also that protein levels of S100A8 and S100A9 are downregulated in response to JQ1 in OCI-AML3. In bone marrow samples of 17 AML patients with different cytogenetic profiles, the relative expression of S100A8 and S100A9 was found to be variable amongst the samples but also in comparison to OCI-AML3 cell line. In further experiments using AML patient bone marrow samples, treatment with JQ1 showed suppression of S100A8 and S100A9 in some patient samples but enhanced expression in other bone marrows. In peripheral blood samples of healthy volunteers, we found that treatment with JQ1 showed notable suppression of both S100A8 and S100A9 with a greater suppression being observed in the monocytic fraction of the samples.

Summary/Conclusions: Our data suggests that JQ1 regulates the expression of S100A8 and S100A9 in AML. The variability of the response seen amongst AML patient samples and AML cell lines may be reflective of the genetic profiles drifting in the clinical samples in which further work may give more detailed insight into the mechanisms of action and potential use of S100A8 and S100A9 in AML prognostic markers.

SUCCESSFUL COVERAGE OF DIFFICULT TO SEQUENCE GENES (CALR, CEBPA, AND FLT3) ASSOCIATED WITH MYELOID DISORDERS USING A HYBRIDISATION-BASED ENRICHMENT APPROACH PRIOR TO NEXT-GENERATION SEQUENCING
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Background: The application of short read NGS for research into myeloid disorders such as myeloproliferative neoplasms (MPNs) and acute myeloid leukemia (AML) has been limited by the inability for certain DNA regions or genes to be reliably targeted. The inability to target DNA regions that can impact the quality of the gene data generated, e.g. large indels and low complexity regions (CALR), high GC content (CEBPA), and complex repetitive elements (FLT3).

Aims: To determine whether a hybridisation-based enrichment approach overcomes the difficulties associated with these genes, and permits the generation of high quality (sufficient de-duplicated depth) data to allow these targets to be accurately interrogated.

Methods: We utilised a hybridisation-based enrichment approach for library preparation in combination with a SureSelect myPanel™ NGS Custom AML panel. The library was then sequenced using a 2x150 bp read length protocol on an Illumina MiSeq®.

Results: Here we present the coverage and variants generated from numerous research samples for each of these difficult to sequence genes. The results clearly show that this approach can reliably detect and accurately size (including low allele frequency) insertions and deletions of up to 52 bp in CALR (exon 9), SNVs and deletions in CEBPA with a de-duplication depth in excess of 2000x as well as ITDs of between 24 and 201 bp in FLT3.

Summary/Conclusions: This approach is suitable for the analysis by NGS of these difficult genes and therefore removes the requirements for supplementary approaches to analyse these difficult genes, such as Sanger sequencing (CEBPA) and fragment analysis (CALR and FLT3).

ASSOCIATION OF miRNA EXPRESSION PROFILES WITH FUNCTIONAL AND MOLECULAR ACUTE MYELOID LEUKAEMIA CATEGORIES
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Background: Development of high-throughput technologies such as Next Generation Sequencing (NGS) allowed the identification of recurrent mutated genes in Acute Myeloid Leukemia (AML), and new molecular markers which help refine patients’ classification in different risk groups.

Epigenetic alterations such as aberrantly expressed microRNAs (miRNAs) also play a significant role on the pathogenesis of AML miRNAs control processes such as cell development, differentiation, proliferation and apoptosis. Therefore, aberrant miRNA expression can affect signaling and metabolic pathways, directing cancer cell biological behavior.

Recently, several studies have classified AML according to different criteria. One example is the recently proposed classification by TCGA, which could cooperate in the development of this hematologic malignancy.

Aims: Our aim is to explore the miRNA profile of NK-AML and to find expression profiles associated with the categories proposed by TCGA and Papaemmanuil et al. Associations of miRNA expression profiles with altered categories could help understand the molecular mechanisms that lead to leukemogenesis.

Methods: CD34+ cord blood progenitor cells from 5 healthy donors and 7 CD34+ NK-AML samples with >70% blasts were obtained. Total RNA from these samples were hybridized onto an Array miRNA 3.0 chip (Affymetrix) in order to identify deregulated miRNAs. The most deregulated miRNAs were validated by qRT-PCR (miScript) in an independent cohort of 73 patients. Muta- tional analysis was performed by Next Generation Sequencing using the AML Community Panel with the Ion Torrent System (Life Technologies).

Summary/Conclusions: We used AML cell line including OCI-AML2, OCI-AML3 and THP-1 in addition to AML patient bone marrow samples and healthy volunteer samples. We carried out RT-qPCR and immunocytochemistry and western blotting techniques to look at levels of S100A8 and S100A9 in samples.

Results: Here we present a profile of 6 miRNAs up-regulated and 61 miRNAs down-regulated in NK-AML vs CD34+ cells. Validation by qRT-PCR confirmed that miR-494 (p=0.028) and miR-495 (p=0.035) were up-regulated in AML and miR-27b (p=0.022), miR-99a (p=0.001), miR-146b (p=0.031), miR-15b (p=0.006) and miR-20b (p=0.001) were down-regulated in NK-AML. Interestingly, some of the deregulated miRNAs were significantly associated to a functional category according to the TCGA classification. Therefore miR-146b was down-regulated in AML with mutations in myeloid transcription factors (p=0.025). Low expression levels of these miRNA cause activation of the factor signaling pathway, which increases transcription. miR-4668 was down-regulated in AML with mutations in activation pathways genes (p=0.004), several target predictors propose RASGEF1A and BRAF as targets of this miRNA. Thus, under-expression of this miRNA could cooperate with mutations leading to the activation of signaling pathways. Regarding to Papaemmanuil’s molecular classification, miR-494 was up-regulated in IDH2-R172 category (p=0.009). High levels of this miRNA are associated with lower expression of TET, specially TET1. Therefore, high levels of miR-494 could contribute to the hypermethylation signature of IDH (AML subtype).
Methods: AML cell lines Kasumi-1, THP-1, MVA-11, U937 and OCI-AML3 were treated in vitro with HF at concentrations ranging from 25 to 1000 ng/ml. The % of apoptotic cells, the distribution of cells in different cell cycle phases, and the HF IC50 was determined for each cell line. We used the Proteome Profiler™ Array – HumanPhospho-Kinase Array to verify the possible tyrosine kinases and signaling pathways that could be modulated by HF. To analyze the in vitro effect of HF, we transplanted the cell lines Kasumi-1 and THP-1 into NOD.Cg-Pkdcr(129gmr1mWj)/SzJ (NSG) mice, which were then treated with intra-peritoneal injections of HF at a dosage of 150 mg/Kg daily for 14 days. The leukemic infiltration of the peripheral blood was quantified by flow cytometry every 2 weeks (using a anti-human CD45.1).

Results: HF IC50 values ranged from 125.58 ng/ml in Kasumi-1 to 786.15 ng/ml in THP-1 cells. Kasumi-1 cells halted in the S phase of the cell cycle when treated with HF, displaying a significant decrease in proliferation, while no effect was observed for THP-1 cells. Corroborating our in-vitro observation indicating resistant of THP-1 cells towards HF, we did not detect significant differences in cell survival of THP-1 cells treated with HF and vehicle control (HF vs vehicle, p=0.02). In contrast, the mean OS for NSG mice transplanted with Kasumi-1 cells treated with HF was significantly prolonged compared to the control group (144 versus 94.5 days; p= 0.007). The proteomic analysis identified significant decrease upon treatment with HF of four phosphorylated-proteins in both cell lines: Phospholipase C gamma 1 (PLCγ1), Proline-rich tyrosine kinase 2 (PYK2), Endothelial nitric oxid synthase (eNos) and Signal transducer and activator of transcription 3 (STAT3 Y705), thus suggesting that these proteins are primary targets of HF. In addition, the protein target of rapamycin (TOR) was downregulated only in THP-1, while the levels of STAT3 S727 and STAT5a/b were significantly decreased by HF treatment only in Kasumi-1 cells. This comparative analysis suggests that the sensitivity to HF may be dependent on inhibition of STAT3/5 pathway.

Summary/Conclusions: In summary, our results suggest that HF may be effective against core binding factor leukemias and, that the methodology based on a Phospho-Kinase Array is useful to identify drug molecular targets.

PB1663
DNA METHYLATION AND HYDROXYMETHYLATION PROFILING IS CAPABLE TO DISTINGUISH AML SAMPLES WITH DISTINCT MUTATIONS IN DNA METHYLATION REGULATORY GENES

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Background: Ablarrant DNA methylation as well as hydroxymethylation is a hallmark of acute myeloid leukemia (AML). Mutations of DNA methylation regulatory genes (DNMT3A, IDH1, IDH2 and TET2) are present in approximately 40-50% of AML. These mutations are often present together with the exception of TET2 and IDH1/2 as well as IDH1 and IDH2, which are usually mutually exclusive.

Aims: We aimed to perform DNA methylation, hydroxymethylation and gene expression profiling in clearly defined subgroups of AML patients with distinct mutations in DNA methylation regulatory genes to see whether there is a clear epigenetic landscape signature.

Methods: We accomplished DNA hydroxymethylation and methylation profiling in 12 AML samples at diagnosis and in CD34+ cells of 3 healthy controls by MethylationEPICarray (Illumina) covering aprox. 850 000 CpGs. AML samples were chosen based on their mutational status and divided into 4 groups: DNMT3A+ (n=3), IDH1+ (n=3), DNMT3A+/IDH1+ (n=3) and IDH2+ (n=3). The remaining DNA methylation regulatory genes as well as CEBPA were unmethylated. 1 μg of genomic DNA was treated with TrueMethyl Seq kit (CEGX) to convert DNA through oxidative bisulfite (oxBS) and bisulfite (BS) treatment. This approach allows us to determine whether CpG is methylated or rather hydroxymethylated in IDH1+ samples are enriched for genes from HOX gene family (P<0.0001) and that these genes are often hypomethylated in DNMT3A+ (n=3), IDH1+ (n=3), IDH2+ (n=3) and CD34+ (n=3) samples relative to CD34+ normals. Clustering of DNA hydroxymethylated in IDH1+ samples were enriched for genes hydroxymethylated in IDH1+ patients were enriched for genes involved JNK cascade (comprising of evolutionarily conserved MAP kinases). The gene expression data did not reveal any cluster coherent with mutational subgroups, only CD34+ normals clustered together.

Summary/Conclusions: We explored that AML patients with clearly defined mutational background exhibit distinct DNA methylation as well as hydroxymethylated gene expression profiles. The presence of two mutations that have the opposing effect on DNA methylation pattern (DNMT3A and IDH1) is linked to mixed DNA methylation patterns, which prevents unambiguous assignment to one cluster. Further, our data support that IDH1+ and IDH2+ represent distinct biological entities. On the contrary, gene expression profile did not support separation of samples into different mutational subgroups. We plan to enlarge the patient’s cohort and validate the most promising genes involved in selected pathways.

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existing hypomethylating-based protocols: a) high gene specificity b) lower cytotoxicity and c) absence of drug based off-target side-effects. In the short term, this research can lead to the identification of novel key regulators of leukemogenesis and new targets for therapeutic treatments; in the long term pave the way for development of RNA-based gene demethylating agents for cancer treatment.

PB1665

**JQ1 AND CURCUMIN COMBINED TREATMENT SHOWS SYNERGIC EFFECTS IN MLL-REARRANGED LEUKEMIA CELL LINES**

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**Background:** MLL-rearranged leukemia accounts for 70% of infant and 10% adult acute leukemias, featuring a particularly poor prognosis and high risk of relapse. Our main field of study is AML, in which nearly 50% of total cases accounts for t(9;11) translocation, the remaining 50% predominantly includes t(6;11)(q27;q23), t(10;11)(p12;q23), t(11;19)(q23;p13.1) and t(11;19)(q23;p13.3). A 2% of AML total cases, however, is characterized by t(4;11) translocation, which is a marker of bad prognosis and it’s, so far, poorly characterized. A key feature of MLL-rearranged leukemia is cMyc overexpression, a well-known oncogene involved in several types of cancer. JQ1 is a novel molecule, which prevents cMYC expression binding an important bromodomain protein, BRD4. Moreover, Curcumin, a natural compound, inhibits HATs enzymes preventing lysine 14 acetylation on histone H3 (AcH3K14), a specific residue which is binding by BRD4 to exert its function.

**Aims:** We would like to explore a potential synergic effect between JQ1 and Curcumin molecules in the attempt to develop a novel therapeutic alternative to standard chemotherapy and to deeply investigate features underlying the molecular pathogenesis in pediatric MLL-rearranged pediatric AML.

**Methods:** Four human leukemia cell lines with MLL fusion protein have been employed in this study: RS4-11, MV4-11 expressing MLL-AF4 and THP1, MOLM13 expressing MLL-AF9 fusion genes. 5′M and 10′M Curcumin were used to treat MLL-4F and MLL-AF9 cell lines respectively, while 250 mM JQ1 was used to treat all the cells lines. After 2 days of treatment, either with single and combined drugs, cell number quantification, based on metabolic activity, was detected through XTT assay. In order to assess the cMYC, CDKN1A, BCL2 transcripts levels and mir-99a expression a quantitative RT-PCR analysis was carried out, while we used western blotting to detect the expression of cMYC, PARP, Caspase3 and AcH3K14. Apoptosis and cell cycle were evaluated by flow cytometric analysis.

**Results:** In apoptosis analysis, a synergic effect was detected for all 4 cell lines, similarly cell cycle evaluation showed a significant accumulation of cells at SubG1 phase (2-8 fold) (Figure 1). XTT metabolic assay showed a reduction in proliferation percentage: 65±5% for curcumin and JQ1 single treatment and 59±5% for combination of drugs in both MLL-AF4 cell lines, meanwhile in MOLM13 cells it was 64±2 and 87±2% for curcumin and JQ1, respectively and 78±2% for their combination (P<0.005). The THP1 cells did not show a significant modulation in the proliferation. We decided to focus our study on t(4;11) translocated cells, considering the more intense effect of the combined drugs on previous analysis. qRT-PCR and western blot experiments revealed a synergic effect of the 2 experimental drugs on both apoptosis and proliferation gene related (bcl2, caspase3, Parp, cdkn1a) as well as on the affect targets of the drugs (cMyc, AcH3K14). Finally, in MLL-AF4 cell lines, curcumin and JQ1 together induced a significant decrease in mir-99a expression.

**Summary/Conclusions:** Our data demonstrated that curcumin and JQ1, inhibiting HATs and BRD4 respectively, exert a more intense synergic effect on MLL-AF4 lines than in MLL-AF9 cells. Increased apoptosis together with a reduced proliferation rate, prompted us to investigate on molecular pathway in which targets of these drugs are involved. Intriguingly, we found a significant decrease in cMyc, bcl2 and AcH3K14 expression, confirming that both curcumin and JQ1 have a synergic effect. Additionally, we revealed a significant reduced expression of mir-99a, a well know oncomiR reported to act as negative regulator of differentiation and involved in drug-resistance, typically up-regulated in pediatric AML and ALL.

PB1666

**TP33B AND TP33I EXPRESSION LEVELS IN RELATION TO NPM1 AND CEBPA MUTATIONS**

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**Background:** Acute myeloid leukemia (AML) is a heterogeneous clonal disorder with the presence of diverse genetic abnormalities in hematopoietic stem cells. The most frequent alterations in normal karyotype AML (NK AML) are mutations in exon 12 of nucleophosmin gene (NPM1). Until now 56 different mutations of NPM1 exon 12 have been described, mostly insertions. The NPM protein plays an important role in cell cycle and apoptosis control. It cooperates with several proteins, among them with p53 and ARF. The median levels of functional nuclear p53 protein are reduced in NPM1 and FLT3 ITD mutant samples. TP53 encodes a tumor suppressor protein which consists of transactivation, DNA-binding and oligomerization domains. Due to alternative splicing it may exist in 13 different isoforms. Alternative splicing of intron 9 leads to production of 2 different proteins, p53β and p53γ, without oligomerization domain (stop codon is localized in exon 9b). These isoforms can be present in acute myeloid leukemia (AML) cells, p53β binds to BAX promoter and can induce apoptosis independent from p53 wt. p53δ has influence on activation of CEBPA which is associated with cell cycle regulation, especially cell cycle arrest and plays also role in cell differentiation. Generally, it is a transcription factor expressed during myeloid lineage development, from progenitor cells to mature granulocytes. Various mutations of CEBPA gene are described. Among them N-terminal and C-terminal mutations, mostly insertions and deletions, are often present.

**Aims:** The goal of the study was to assess mutational status of NPM1, CEBPA and FLT3 in association with TP53beta and TP53gamma expression levels.

**Methods:** 75 NK AML patients were included in the study. NPM1, CEBPA and FLT3 gene mutations were analyzed by direct sequencing. TP53β and TP53γ expression levels were assessed by real time PCR. Expression levels were analyzed with ΔΔCt method, with ABL as a control gene and K562 cell line as a calibrator.

**Results:** In all 75 cases, TP53β and TP53γ transcripts were detected. 36 patients had NPM1 mutations, 25 had CEBPA mutations or known polymorphisms, and 25 had FLT3 ITD mutation. Assessed median expression level of TP53β was much higher (ΔΔCt 43,11) than TP53γ (ΔΔCt 10,85; p<0,05). Furthermore, expression level of TP53γ in CEBPA mutated group (ΔΔCt 11,4) was significantly lower than in CEBPA wt group (ΔΔCt 17,7) (p=0,03). We have not found any other important correlation between mutations of studied genes and TP53β or TP53γ expression. We also classified patients, according to median expression value of TP53, to two groups: with overexpression or with low expression. Haematological and clinical features, such as white blood cells count (WBC), blasts count in bone marrow or patient age did not depend on TP53 isoform expressions. However, statistical analysis showed important difference between WBC count in NPM1mutated and NPM1wt groups.

**Summary/Conclusions:** Obtained results may suggest a clinical importance of simultaneous analysis of TP53 isoform expression and mutations in CEBPA gene. It may be hypothesized that a changed sequence of the latter gene might influence TP53 isoform expression and in consequence regulate the cell cycle.
Acute myeloid leukemia (AML) is a malignant disorder of hematopoietic stem and progenitor cells (HSPCs), characterized by the accumulation of immature blasts in the bone marrow and peripheral blood (PB) of affected patients. Standard induction therapy, based on cytosine arabinoside and an anthracycline, leads to complete remission in approximately 50% to 75% of patients, depending on prognostic factors, such as age or the presence of certain gene or chromosomal changes. In spite of favorable primary response rates, only approximately 20% to 30% of the patients enjoy long-term disease survival.

Aims: Our aim was to compare the protein expression profile of peripheral blood mononuclear cells (PBMCs) of AML patients at time of diagnosis and after induction therapy.

Methods: PB samples were taken from seven AML patients in Medellin-Colombia before and after concluding the induction therapy. Informed consent was obtained prior to sample collection, PBMCs were isolated from the 14 blood samples using a Histopaque-1077 solution. Cells were resuspended in lysis buffer (0.5% Triton x-100, 50 mM Tris-HCl pH 8.0, 150 mM NaCl, 1 mM EDTA, protease inhibitor) and proteins precipitated with trichloroacetic acid. Protein pellets were separated by 2D SDS-PAGE (pL ~ 10 NL), and stained with SYPRO®Ruby. The proteomes were compared using PDQuest™ Advanced 8.0.1 Software. Protein spots of interest were identified by MALDI-TOF-MS/MS sequencing.

Figure 1. Summary/Conclusions: CEBPAdm cases showed an homogeneous immunophenotype with positivity for CD45, CD7, CD34, CD123, CD17, HLA-DR, CD71, CD33, CD13 and CD15. CD63 and/or CD56 overexpression was detected in a subgroup of cases (9/39, 23%) with an adverse outcome. The current findings suggest that CD36 and CD56 reactivity should be investigated in larger series of CEBPAdm AML cases. Small leukemic populations with B-cell markers are not uncommon in CEBPAdm AML (3/39, 7%).

PB1669

PROTEOME CHANGES IN ACUTE MYELOID LEUKAEMIA PATIENTS BEFORE AND AFTER INDUCTION TREATMENT

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Background: Acute myeloid leukaemia (AML) is a malignant disorder of hematopoietic stem and progenitor cells (HSPCs), characterized by the accumulation of immature blasts in the bone marrow and peripheral blood (PB) of affected patients. Standard induction therapy, based on cytosine arabinoside and an anthracycline, leads to complete remission in approximately 50% to 75% of patients, depending on prognostic factors, such as age or the presence of certain gene or chromosomal changes. In spite of favorable primary response rates, only approximately 20% to 30% of the patients enjoy long-term disease survival.

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Results: Image analysis revealed an average of 464 protein spots in PB samples taken at time of diagnosis, and an average of 346 spots in PB taken after induction therapy, reflecting changes in protein expression due to treatment. Comparing the proteomes, we found 11 spots that differed significantly (fold change of +/- 1.5 and p < 0.05). Of these, seven proteins were up-regulated and four were down-regulated at time of diagnosis (before treatment) compared to after induction treatment. Nine of these spots corresponded to low molecular weight proteins (<40 kDa) and 2 spots have a molecular weight between 40-60 kDa. Based on the molecular weight and isoelectric point information of these spots we were able to search for proteins reportedly involved in leukemia, in order to propose possible identities (see Table 1). In terms of biological processes, four proteins (eIF5B, HSP27, 14-3-3 protein zeta/delta, and GST-P) are involved in the regulation of apoptosis. The F-actin-capping protein subunit beta could also be of interest, as reorganization of F-actin reflects unique characteristics of the differentiation process in promyelocytic leukemia cells. RuvB-like 2 is a positive regulator of histone acetylation and DNA repair. GRBP2 is a protein involved in the MAPK cascade and regulation of PI3K signaling, pathways regulating diverse cellular functions altered in leukemogenesis such as proliferation, differentiation, and apoptosis. Alpha-enolase is a key glycolytic enzyme; however, it has been shown to be a multifunctional protein involved in cancer. It promotes cell proliferation by also regulating the MAPK and PI3K pathways. Transaldolase is part of the pentose-phosphate pathway. Annexin II acts in angiogenesis and has multifaceted role in human health and disease.

Summary/Conclusions: The protein expression profile of AML patients changed after induction treatment. We found 11 spots that differed significantly, and propose possible identities for these. Further analyses are pending in order to experimentally establish the identities and correlate with response to treatment.

PB1670

AMP-ACTIVATED PROTEIN KINASE ACTIVITY INTERFERES WITH OVEREXPRESSION OF NUCLEOPHISMIN IN CYTARABINE-INDUCED CHEMORESISTANT AML CELLS

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Background: Cytarabine is a chemotherapeutic drug used alone or in combination with other anticancer drugs to treat acute myeloid leukemia (AML). New treatment strategies are emerging to enhance the anti-cancer effect and decrease the toxicities. Nucleophosmin (NPM1 or B23) is a ribosomal protein located mainly in nucleolus, and multifunctional enzyme in cancer cell growth and proliferation. AMP-activated protein kinase (AMPK) is a critical energy sensor to regulate homeostasis and plays a potential role for anti-cell proliferating activity.

Aims: We investigated the effects of AMPK activation on the cell death (apoptosis) and proliferation of AML cells treated with low or high concentration of cytarabine, an anti-leukemic drug, to predict the mechanisms responsible for AML cells chemoresistance.

Methods: The HL-60 (FAB M2) cells were exposed to the different drug combinations including cytarabine and AMPK activators. The molecular mechanisms of AMPK activation by cytarabine were investigated by western blotting of p-AMPK level. Cell viability and apoptosis were assessed using cell counting kit-8 assay and flow cytometry.

Results: We found that cell apoptosis (36.27 ~ 42.11%) showed little dependence on cytarabine concentrations (10, 100, and 1000 mM), while the overexpression of NPM1 overexpression level increased proportionally with drug dependence, indicating the drug-induced cell resistance. In the same point, cytarabine also inhibited the phosphor-activity (Thr172) and expression level of AMPK, which has mTOR-p70S6K pathway-repressor activity. As expected, single cytarabine treatment increased the ratio of p-mTOR/mTOR and p-p70S6K/p70S6K. Co-treatment of AMPK activator (phenformin or AICAR) in cytarabine-treated HL-60 AML cells inhibited significantly the induction of NPM1 overexpression level with the decrease of phosphor-activities of mTOR and its substrate p70S6K, resulted in the accelerated cell apoptosis.

Summary/Conclusions: Our results suggest that the higher concentration of cytarabine induces NPM1 overexpression, and that AMPK activation might be used to sensitize AML cells to cytarabine with the control of NPM1 expression levels. These modulations to standard therapeutic strategies could actually enable the reduction of the chemotherapeutic dose, therefore reducing their toxicity and adverse effects.

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PB1671

QUERCETIN REGULATES TELOMERE-BINDING PROTEINS EXPRESSION OF POT1, TRF1, TRF2 TO INHIBIT PROLIFERATION AND INDUCE APOPTOSIS IN AML THP-1 CELLS

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Background: Leukemia cells are limitless cell sources for initiation and maintenance of leukemia. Telomere-binding proteins are key regulators of telomere characteristics of the differentiation process in promyelocytic leukemia cells. It promotes cell proliferation by also regulating the MAPK and PI3K pathways. Transaldolase is part of the pentose-phosphate pathway. Annexin II acts in angiogenesis and has multifaceted role in human health and disease.

Summary/Conclusions: The protein expression profile of AML patients changed after induction treatment. We found 11 spots that differed significantly, and propose possible identities for these. Further analyses are pending in order to experimentally establish the identities and correlate with response to treatment.

PB1672

PPARγ AGONISTS INHIBIT ADHESION SIGNAL TO ENDOTHelial CELLS IN THE DIFFERENTIATION INDUCTION OF 15d-PGJ2 ACUTE PROMYELOCYTIC LEUKEMIA CELLS.

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Background: All-trans retinoic acid (ATRA) has successfully been used in the treatment of acute promyelocytic leukemia (APL) patients, with a remission rate of greater than 90%. Despite the high cure rates, induction mortality is a still a problem in APL. One of the most common causes of death was the differentiation syndrome (DS). The early administration of high-dose dexamethasone at the onset of the first
Acute myeloid leukemia - Clinical

PB1673

IN VITRO DRUG SENSITIVITY TEST IN THE INDIVIDUALIZED ANTI-LEUKEMIA CHEMOTHERAPY FOR THE NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA


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Background: The biological properties, genetic abnormalities of leukemic cells influence on their sensitivity to chemotherapeutic drugs. It is widely known that there can be significant differences both in genetic features as well as in drug resistance profile of individual tumors with the same phenotype.

Aims: The purpose of this study was to analyze the relationship between in vitro chemosensitivity test results using the Cell Titer-Glo assay and clinical response on chemotherapy, and to find the possibility of optimizing the treatment for individual patients according to their actual drug resistance.

Methods: For The Cell Titer-Glo assay, we obtained bone marrow aspirates or peripheral blood samples from 68 patients with newly diagnosed acute myeloid leukemia at the time of initial diagnosis. The following drugs were tested: cytarabine, daunorubicin, idarubicin, fludarabine, etoposide, and methotrexate. We evaluated clinical response and survival outcome according to chemosensitivity of drugs and protein expression.

Results: In this study, in vitro chemosensitivity test with the Cell Titer-Glo assay showed the relationship between chemosensitivity and survival outcome significantly. The 5-year overall survival rates with dichotomized chemosensitivity of idarubicin (64.6% vs 33.3%, p=0.046), cytarabine (63.1% vs 43.3%, p=0.0291), and fludarabine (80.1% vs 37.5%, p=0.020) were higher in low concentration level than in high concentration level. There was a tendency of higher relapse-free survival rate at 4-year in the patients with low level IC50 than in the high level IC50. However, cytotoxic effect of testing drugs in vitro by the Cell Titer-Glo assay did not show a relationship with complete remission rate after induction and leukemia recurrence rate.

Summary/Conclusions: Although the Cell Titer-Glo assay did not provide the prediction of clinical response of induction treatment, it can be a useful tool in individually optimizing the chemotherapy of patients with newly diagnosed acute myeloid leukemia.

PB1674

PROGNOSTIC IMPACT OF P53 EXPRESSION IN BONE MARROW BIOPSY OF PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Several studies have shown that the presence of the TP53 mutation is related to an unfavorable prognosis in patients with acute myeloid leukemia (AML). However, there are few reports on the evaluation of its expression by immunohistochemistry in bone marrow (BM) biopsy.

Aims: To evaluate the expression of p53 in BM biopsy of AML patients at diagnosis and its impact on survival.

Methods: This retrospective analysis included 85 patients with de novo AML diagnosed from January 2005 to December 2015 submitted to BM biopsy at diagnosis. p53 expression was detected by immunohistochemistry, and staining was evaluated using the H-score (range 0-300). The t-Test and Mann-Whitney U test were used to detect differences in the distribution of continuous parametric and nonparametric variables, respectively. Overall survival (OS), disease-free survival (DFS) and event-free survival (EFS) were calculated using the Kaplan-Meier method. The log-rank test was used for comparison of survival curves. The interaction between the examined prognostic variables was tested with univariate and multivariate Cox regression analysis.

Results: Median age was 60 years (17-81). There was a predominance of patients >50 years (54.1%) and males (56.5%). The median H-score for p53 was 11.8 (0.4-161.1), with no significant correlation with age or cytogenetic risk. p53 expression was significantly higher in patients with a complex karyotype (p=0.0031) and high risk by European Leukemia Net (ELN) criteria (p=0.047).

There was a positive correlation with complex karyotype and prognostic risk by ELN. Excluding early deaths (<30 days from induction), patients younger than 60 years with H-score >60 showed worse overall survival when compared with patients with H-score <60 (0% vs 14.6%, respectively) (p=0.048). There was no statistical difference in disease-free survival and event-free survival. In the Cox univariate analysis including all cases, peripheral leukocyte counts at diagnosis (p=0.014), cytogenetic risk groups (p=0.07), ELN risk categories (p=0.023) and H-score (p=0.025) were significant. In a multivariate model including leukocytes, ELN risk and p53, all variables remained in the model.
Summary/Conclusions: Expression of p53 assessed by immunohistochemistry is a fast, sensitive and promptly available tool for prognostic evaluation of AML. A high expression of p53 (H-score >60) was related to a lower overall survival in de novo AML.

PB1675

Abstract withdrawn.

PB1676

LONG-TERM FOLLOW-UP OF SALVAGE TREATMENT FOR RELAPSED AML WITH CLADIREBINE, HIGH DOSE CYTARABINE AND IDARUBICIN

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Background: Despite improving response rates in induction treatment for AML during the last years the outcome for relapsed or refractory AML is still poor. Currently, no standard therapy exists for patients with relapsed AML. Furthermore, CR rates are lower than in newly diagnosed patients and range between 15% and 50%. There is evidence that cladribine in AML as well as an enhancing effect on other cytostatic drugs such as cytara- bine (Arac) and thus may help to overcome resistance mechanisms.

Aims: Therefore, testing the combination of 2CdA, Arac and idarubicin (CAI) seems reasonable. Here we present the final analysis from our single-center phase II trial evaluating the safety and efficacy of CAI in relapsed AML patients after a follow-up of 5 years.

Methods: Patients with relapsed AML after at least 6 months of remission and COG 0-2 were included. Chemotherapy regime consisted of two courses of 2CdA 5 mg/m²/d, h-1, d-3, Arac 1000 mg/m²/d, h-1, d-3 and idarubicin 8 mg/m²/d, d-3. After 8 patients, the prolonged duration of neutropenia especially in course 2 prompted us to change the protocol by 1 application of growth factors from day 15 onwards, and 2) omission of idarubicin from the 2nd course. The primary endpoint was the overall remission rate and safety of CAI.

Results: Because of slow recruitment the study was stopped after 20 patients. The median age was 63 years. 40% were female. 19/20 (95%) patients were included in the first relapse after at least 6 months of CR following 1st line ther- apy for AML. 1/20 (5%) patient was included with a second relapse. In 14/20 patients cytogenetic data at the timing of relapse were available, according to the ELN-risk-group 2017 3/14 (22%) had favorable cytogenetic changes, 9/14 (64%) intermediate and 2/14 (14%) belonged to the adverse cytogenetic group. The performance status was good in most patients (ECOG 0 in 10%, ECOG 1 in 80%), but reduced (ECOG 2) in 2 (20%) patients. After the first course, CR/CRi was achieved in 60% and PR in 10% of patients. Median duration of neutropenia was 19-41 days. Duration grade 3 or 4 neutropenia was 24 days (range 18-41d). The main grade 3 or 4 non-haema- tologic toxicity was infection seen in 85% of courses. Nausea occurred in 30%, hepatotoxicity, mucositis and diarrhea in 11% of courses. Cardiac or renal tox- icities grade 3/4 were not observed. Two patients (10%) died due to infection. Six patients received a second course of CAI/CA. Altogether, 6 patients were refractory. Nine patients (48%) proceeded to allogeneic stem cell transplantation after induction therapy with CAI. Of those, 4 patients are still alive and free of leukemia and one patient died in CR 88 months after salvage-therapy accounting for a 5-year survival rate of 55%.

Summary/Conclusions: Combination therapy with CAI in relapsed AML patients is feasible and induces good response rates. Combined with allogeneic stem cell transplantation, long-term survival can be achieved. However, infec- tion rates are a serious complication warranting intensive supportive care.

PB1677

HIGH EVI1 EXPRESSION PREDICTS POOR OUTCOMES IN ADULT ACUTE MYELOID LEUKEMIA PATIENTS WITH INTERMEDIATE CYTOGENETIC RISK RECEIVING CHEMOTHERAPY ONLY

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Background: Nearly half of acute myeloid leukemia (AML) patients are defined as an intermediate cytogenetic risk, however those patients in the group have greatly varied outcomes and need to be stratified. Apart from gene mutation, abnormal gene expression might also be prognostic, and ecotropic viral integration site 1 (EVI1) expression is a representative. To date, the poor prognostic impact of EVI1 expression in AML has been reported, but almost all studies have been undertaken by European researchers. EVI1 prognostic significance in AML remains to be confirmed in other populations. Furthermore, because the selection protocol and cutoff value selection methodologies differed among studies, the threshold for defining EVI1 high expression remains obscure, which hinders its clinical routine application.

Aims: We investigated the prognostic impact of EVI1 transcript levels in Chi- nese adult intermediate cytogenetic risk AML (iC-AML) patients who received chemotherapy only in a single center. The appropriate cutoff values for grouping EVI1 expression were also evaluated.

Methods: A total of 191 adult patients receiving chemotherapy only were includ- ed in this study. They were diagnosed as iC-AML according to morphology, immunophenotyping, cytogenetics and molecular biology. Their bone marrow samples were collected at diagnosis. Real-time quantitative PCR was performed to test EVI1, MLL partial tandem duplicate (MLL-PTD) and WT1 tran- scripts, and their transcript levels were calculated as the percentage of target transcript copies/ABL copies. NPM1 mutations and FLT3 internal tandem dupli- cation (FLT3-ITD) were individually screened by real-time quantitative PCR and quantified as the percentage of copies per million AML blasts. All patients were simultaneously tested EVI1, MLL-PTD and WT1 transcripts. All partici- pants provided written informed consent in accordance with the Declaration of Helsinki.

Results: The upper limit of EVI1 transcript levels in 27 NBM samples was 8.0%. Receiver operating characteristic curve analysis showed that 1.0% (a 9.0-log reduction from the normal limit) was the EVI1 optimal diagnostic cutoff value for significantly differentiating relapse (P=0.049). A total of 23 patients (12%) had EVI1 levels ≥1.0%. EVI1≥1.0% had no impact on complete remission achieve- ment. EVI1≥1.0% was significantly associated with lower 2-year relapse-free survival (RFS), disease-free survival (DFS) and overall survival (OS) rates in the entire cohort (P=0.0003, 0.0017 and 0.0009), patients with normal karyotypes (n=148, P=0.0032, 0.0047 and 0.0007) and FLT3-ITD (-) patients (n=150, all P<0.0001). Multivariate analysis showed that EVI1≥1.0% and FLT3-ITD (+) were independent adverse prognostic factors for RFS (Table 1), DFS and OS in the entire cohort. In addition, patients with EVI1 between 1.0% and 8.0% had 2-year RFS rates similar to those with EVI1≤0.8% (P=0.16), and both patient groups had significantly higher RFS rates than those with EVI1≥1.0%.

PB1678

EFFICACY AND SAFETY OF DECITABINE IN ELDERLY AML PATIENTS: A REAL LIFE MULTICENTER EXPERIENCE OF THE NETWORK RETE EMATOLOGIA LOMBARDICA

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Background: The optimal treatment decision in older patients (pts) with AML remains controversial, especially in patients pts with comorbidities, non-fit to intensive therapy or with AML adverse biologic features. Recently decitabine was approved in Italy in AML pts unfit to chemotherapy aged ≥65 years (y) and could be adopted in a population based setting.

Aims: To evaluate efficacy and toxicity of decitabine in a consecutive series of elderly AML pts (no M3), considered unfit to chemotherapy (CT) according to Ferrara et al (Leukemia, 2013) and treated at 6 centers of the Hematological Lombardy Network (REL).

Methods: Between Dec 2015 and Dec 2016, 46 (F/M: 22/24) newly diagnosed AML pts, not eligible for CT, and/or receiving intensification therapy were treated at 6 centers of the Hematological Lombardy Network. All pts were ≥65 yrs old (n=46). The primary endpoints were overall survival (OS), time to relapse (TTR) and quality of life (QoL) assessed by a modified EORTC QLQ-C30 questionnaire, and secondary endpoints were the number of adverse events and the duration of stable disease (SD).

Results: The median age was 76 (69-85) yrs. Median OS was 11.2 months (95% CI, 9.3-20.9 months). OS was significantly shorter in patients with M3 compared to other cytogenetic risk subtypes (P=0.003). The median time to relapse was 5.0 months (95% CI, 2.4-5.0 months) and the median duration of SD was 1.9 months (95% CI, 1.5-2.3 months).

Summary/Conclusions: The results of this study confirm the findings of previous studies in with decitabine was well tolerated in elderly AML pts. Combining the results of this study with the results of other studies, decitabine is an effective and safe treatment option for elderly AML pts with comorbidities or unable to receive CT.
PB1679
CLOFARABINE, CYTARABINE AND MITOXANTRONE FOR RELAPSED OR REFRACTORY ACUTE MYELOID LEUKAEMIA – INTERIM RESULTS OF A PROSPECTIVE PHASE 2 STUDY

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Background: In unselected patients with acute myeloid leukaemia (AML) in first relapse or refractory to primary daunorubicin / cytarabine therapy, complete response (CR) rate is merely 20 – 30%. In patients <80-years old, CR rates of about 55% may be achieved. The total number of cycles administered was 231 (median 3.5; range 1-20). In 37/46 evaluable pts (2 ongoing, 1 early and 6 aplastic deaths), overall response rate (ORR) and complete remission (CR) rate were 51.1% and 32%, respectively. Partial response (PR), neutrophil and hematomatoligical improvement were achieved in 5.6% and in 13.6%, stable disease in 29.9% and failure in 19% of pts, respectively. Median time to best response was 3.5 months (range 1-8.5). Median response duration was 5.3 months (1-18+ ms). Relapse/disease progression was observed in 42% of responders. ORR was 21.4%, 47.3% and 77% in adv, NK and intermediately advanced disease, respectively (P=0.0289). After a median follow-up of 6.5 months, median survival was 8.4 months and projected OS at 1 and 2 y was 43%+9 (SEM) and 30%+12 (SEM). Treatment was fairly well tolerated except for a high incidence of infections (46 episodes in 231 cycles) particularly during the first three cycles (29% vs 11%) (p 0.0072). Pneumonia was the most frequent infection (46%), followed by sepsis (28%). It was more frequent during the first 3 cycles [14% vs 4%: p 0.0123] when 44% of cases were of suspected fungal origin (3 probable aspergilliosis and 4 possible IFI). Death occurred in 24 pts (52.2%): 12 (50%) of disease progression, 1 of early CNS hemorrhage and 1 (4.5%) of infection. In the first 3 months, infections were responsible for 46.7% of deaths. Pulmonary IFI were fatal in 57% of these cases. These figures are higher than those reported by Casho (JCO 2010) where the frequency of pneumonia was 11%.

Summary/Conclusions: These preliminary data confirm, in a population based setting, the high efficacy of decitabine and its longer time to response (more than 10 days) compared to CT. However infections complication were more frequent than expected and often fatal, particularly during treatment. Since pneumonia, especially IFI, was the major cause of death, the adoption of routine antimicrobial prophylaxis may be considered in order to reduce early mortality and further improve the results.

PB1680
FATAL EVOLUTION IN THE FIRST 96 HOURS OF PATIENTS DIAGNOSED WITH ACUTE LEUKAEMIA: ANALYSIS OF A SERIES OF 346 CONSECUTIVE CASES OF ACUTE LEUKAEMIA – INTERIM RESULTS OF A PROSPECTIVE PHASE II STUDY

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Background: The very early death of a newly diagnosed acute leukemia (AL) patient is very frustrating, and there are very few published works (except for the case of acute promyelocytic leukemia, APL) analyzing this circumstance and the features of these patients.

Aims: The objective was to study the main characteristics of patients with acute leukaemia who died within the first 96 hours after diagnosis in our centre in the last 15 years.

Methods: We studied all cases of acute leukaemia diagnosed in our institution between April 2002 and January 2017, focusing on the analysis of those who died within the first 96 hours after diagnosis. In this subset of patients, we collected data concerning clinical presentation, hemogram, biochemical parameters, coagulation status, performance of a bone marrow aspirate, acute leukaemia subtype, started therapy, initiation or not of induction chemotherapy, time elapsed from diagnosis to death (hours), and cause of death, among others.

Results: A total of 346 consecutive cases of acute leukemia were recorded in this period of time: 222 of acute myeloid leukaemia (AML, 64%) and 124 of acute lymphoblastic leukaemia (ALL, 36%). Thirty-three patients were diagnosed of acute promyelocytic leukaemia (15% of all AML). Those patients who died in the first four days after the diagnosis were only seven (2%), with a median of 45 hours before diagnosis (range 21-96). The clinical and analytical findings of these 33 patients are shown in the Table 1. They were 5 men and 2 women with a median of 57 years (range 22-91). Two of the seven patients had an APL (6% of all diagnosed APL). All patients showed leukocytosis, but hyperleukocytosis was only recorded in 27 patients, and severe thrombocytopenia (platelet ≤ 20 x 10^9/L) in 37. There was possibility of bone marrow aspiration only in 4/7 cases. Coagulopathy was detected in four of six patients, including criteria for disseminated intravascular coagulation (DIC) in three cases. The exitus took place in the Intensive Care Unit in 5 cases, while it occurred in the Hematology facility in two.

Table 1.

Summary/Conclusions: In our experience, about 2% of patients with acute leukaemia die within the first 96 hours after diagnosis (including 6% of APL). Clinical and analytical features of this subset of patients are very heterogeneous, although AML clearly predominates on ALL. More extensive and multicenter studies are needed to deepen into the circumstances conditioning this early fatal course of the disease.

PB1681
PRIMARY POSACONAZOLE PROPHYLAXIS IN ACUTE MYELOID LEUKAEMIA - A SINGLE CENTER REAL LIFE EXPERIENCE

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Background: Invasive fungal infections (IFI) are a major cause of mortality and morbidity in acute myeloid leukemia (AML) patients receiving remission induction therapy, and relapsed/refractory AML patients. Posaconazole prophylaxis has shown the greatest benefit in preventing IFI in AML.

Aims: We present the data of our real-life experience in AML patients under PP.

Methods: We have retrospectively reviewed the data from 82 AML patients...
receiving 105 cycles of chemotherapy between June 2012 and December 2016 in Marmara University Pendik Research and Training Hospital. Median patient age was 50 years (18-72); and there was no significant gender difference (38 females vs 44 males (46% vs 54%). All patients had active disease, 78 (74.3%) of them received 3+ (darubicine - ara-c), 25 (23.8%) of them FLAG-Ida, 1 patient received EMA and 1 patient received CLARA chemotherapy protocol. Acute promyelocytic leukemia was excluded from the analysis. All patients received posaconazole as oral suspension at the dose of 200 milligrams three times daily starting on the first day of chemotherapy. Prophylaxis was continued until marrow regeneration, or occurrence of IFI, or onset of adverse events, or discontinuation due to other reasons. All fungal infections were classified as possible, probable or proven according to European Organization for the Research and Treatment of Cancer (EORTC) and the Mycoses Study Group (MSG) consensus criteria.

Results: Mean posaconazole prophylaxis duration was 20±13 (1-68) days. This duration was 29.7 days (16-50) in patients receiving prophylaxis until marrow recovery; and 18.9 (9-34) days in patients who did not receive prophylaxis due to adverse events and other reasons. Posaconazole prophylaxis was administered until marrow recovery without IFI (clinical success rate) in 42 of 105 (40%) chemotherapy cycles. In 18 cycles prophylaxis was stopped after diagnosis of IFI (17.1%). Discontinuation of prophylaxis was due to adverse events in 6 cycles (5.7%), and due to other reasons (diarrhea, intolerance of oral medication, recurrent high grade fever, death) in 39 cycles (37.1%). IFI incidence under effective posaconazole prophylaxis was 28.1% (18/64). Total clinical failure rate was 60% (63/105). IFI was diagnosed with pulmonary nodules in 12 of 18 patients (66.6%; EORTC-MSG: probable), with histoplasman positivity in 3 patients (16.6%; EORTC-MSG: probable), and with fungal culture in 3 patients (16.6%; EORTC-MSG: proven). Data from 70 patients were available for mortality analysis. In patients receiving effective posaconazole prophylaxis, all-cause mortality rate at day 100 was (8/44; 20.4%) significantly lower than patients unable to continue posaconazole prophylaxis (12/26; 46.1%) (p=0.023). In the subset of patients receiving prophylaxis as planned; there was no statistically significant difference in IFI incidence between previously untreated AML (13/46; 28.2%) and relapsed/refractory AML (5/18; 27.7%).

Summary/Conclusions: In our real-life experience, we have demonstrated early effective prophylaxis against fungal infections. Although our IFI rate was comparable to other real-life data, our clinical failure rate was slightly higher. This is probably due to compliance issues, since in many chemotherapy cycles (37.1%) posaconazole was discontinued due to “other reasons” such as drug intolerance. Although not as effective as in the clinical trials; our data still supports the use of posaconazole prophylaxis in high risk AML patients.

PB1682

CLINICAL AND PROGNOSTIC VALUE OF FLT3 MUTATIONS IN ACUTE MYELOID LEUKAemia PATIENTS IN ROUTINE CLINICAL PRACTICE – SINGLE CENTER EXPERIENCE

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Background: Detection of FLT3 gene mutations in acute myeloid leukemia (AML) now recognized as an unfavorable factor that affects the disease course, emerging the risk of relapses and overall survival (OS) shortening. Although about 30% of AML patients harbor one of the FLT3 gene lesion, at present there are no internationally standardized assays to quantify FLT3 mutation burden and no results of randomized clinical trials intended to individualize AML treatment based on FLT3 status. Some hematologists advocate to allo-SCT in the case of FLT3-ITD gene mutations; however, the groups were seen only in WBC and blasts. Chromosomal aberrations were revealed in 38% of FLT3-ITD, 64% of FLT3-TKD, none of FLT3-ITD+TKD and 51% of FLT3- patients. All patients received chemotherapy (73±5.2, HAM). Transplantation of hematopoietic stem cells (SCT) was performed in 28 (alloauto 17/11) (14%) patients; FLT3-ITD allo-3; FLT3-TKD allo-1, auto-1; FLT3-ITD allo-13, auto-10. We found significant (p=0.00024) differences regarding OS between FLT3-ITD, FLT3-TKD and FLT3- patients (Figure 1). Median survival times were: 5.1 months for FLT3-TKD, 7.1 months for FLT3-TKD and 13.0 months for FLT3- patients.

Figure 1.

Summary/Conclusions: We confirmed the role of FLT3 gene mutations as an unfavorable factor for AML patients in routine clinical practice by our experience. The investigation of qualitative assessment potential and target therapy value especially in SCT ineligible FLT3 gene mutations positive patients has of great value for AML management.

PB1683

TARGETING ENDOTHELIAL DYSFUNCTION FOR PROTECTION FROM ANTHRACYCLINE-INDUCED CARDIOTOXICITY IN PATIENTS WITH ACUTE LEUKAEMIA AND CO-MORBID ISCHEMIC HEART DISEASE

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Background: Cardiotoxicity of chemotherapeutic drugs, in particular anthracycline antibiotics (AA), is one of the biggest problems in treatment of patients with acute leukemia (AL). Chemotherapy with AA is accompanied by systemic endothelial dysfunction, increasing the cardiovascular toxicity risk and promoting vascular complications. Patients with co-morbid ischemic heart disease (IH) are at extremely high risk of myocardial injury and in need of anthracycline cardiotoxicity (AC) prevention.

Aims: To assess the effectiveness of L-arginine in the prevention of endothelial dysfunction as a predictor of acute AC in patients with AL and co-morbid ischemic heart disease.

Methods: A total of 66 patients with newly diagnosed acute leukemia (acute lymphoid leukemia – 7 patients, acute myeloid leukemia – 59 patients) and co-morbid ischemic heart disease were included in the study. The cohort consisted of 40 (60%) males and 26 (40%) females, age of 54–72 years, ECOG I-II. The duration of IH ranged from 3 to 15 years. Chemotherapy (CT) schemes included AA (doxorubicin). The evaluation of endothelial dysfunction was performed by determining the stable metabolites of nitric oxide – nitrite anions [NO2−] and activity of total NO-synthase in serum of patients before the CT and year after its cessation. To determine effective dose of AA from 100 to 200 mg/m² by doxorubicin. The mean total cumulative dose of AA reached 162,04±24,65 mg/m² and 13.0 months for FLT3- patients.

Figure 1.

Summary/Conclusions: We confirmed the role of FLT3 gene mutations as an unfavorable factor for AML patients in routine clinical practice by our experience. The investigation of qualitative assessment potential and target therapy value especially in SCT ineligible FLT3 gene mutations positive patients has of great value for AML management.
Results: In the debut of AL prior to the CT in all 66 (100%) patients the increased activity of total NOS in 3.8 times compared with the norm (p<0.001) was noted, with simultaneously reduced concentration of \([NO_2^-]\) in 1.5 times relatively normal values (p<0.05) (Table 1). As a result of two CT courses of remission induction in patients of group I the tendency to reduce the total NOS activity compared with its level before treatment was observed. At the same time the significant decrease of \([NO_2^-]\) in 1.8 times relatively normal values (p<0.01) and a trend to lower their content in 1.2 times compared with the data before treatment (p>0.05) was noted. These changes constitute the violation of NO-dependent vasodilation mechanism and endothelial dysfunction intensification. Provided achieving low cumulative dose of AA in patients of group II on the background of AC prevention with L-arginine showed a significant decrease in 1.9 times the total NOS activity (p<0.001) with a simultaneous tendency to increase concentration of \([NO_2^-]\) in 1.3 times (p<0.05) compared to that before treatment.

Table 1.

Summary/Conclusions: Thus, during the CT with the inclusion of AA without L-arginine in patients with AL and co-morbid IHD we observed the depletion of NO synthesis production, accompanied by endothelial dysfunction impairment. The additional appointment of L-arginine on the background of CT can restore synthesis of NO and, respectively, the mechanism of NO-dependent vasodilatation, thus reducing the risk of early anthracycline cardiotoxicity development.

PB1684

CLINICAL CHARACTERISTICS AND SURVIVAL OUTCOMES IN ACUTE ERYTHROID LEUKEMIA (AML-M6): AML/MDS WORKING PARTY STUDY OF KOREAN SOCIETY OF HEMATOLOGY


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Background: Acute erythroid leukemia is a morphologically distinct and rare entity designated as M6 in FAB classification. In Korea, patients with AML-M6 have been treated as acute myeloid leukemia with intensive chemotherapy, accompanied by endothelial dysfunction impairment. The additional appointment of L-arginine on the background of CT can restore synthesis of NO and, respectively, the mechanism of NO-dependent vasodilatation, thus reducing the risk of early anthracycline cardiotoxicity development.

Aims: The aims of this multi-center study were to characterize clinical characteristics and treatment outcomes in patients with newly diagnosed acute erythroid leukemia.

Methods: Clinical data from newly diagnosed M6-AML patients between 2002 and 2012 at 11 academic centers were retrieved from the electronic registry of HSCT. Cytogenetic results were available in 80 patients. Among them, karyotype was normal in 43 (53.8%) and complex in 13 (15.5%) patients, respectively. Trisomy 8 was observed in ten (12.5%) patients. Monosomies of chromosome 5 and 7 were observed in five (6.2%) and four (5.0%) patients, respectively. Forty (5.0%) patients had t(9;22)q34;q11.2. Cytogenetic risk group according to UKMRC criteria were intermediate in 63 (78.8%) patients, and poor in 17 (21.2%) patients. Seventy-two (85.7%) patients received induction chemotherapy and 55 patients (76.4%) achieved complete remission. Nineteen patients received two or three cycles of induction chemotherapy. Thirty-eight patients (45.2%) underwent autologous hematopoietic stem cell transplantation (HSCT): 8 patients, matched-sibling donor; 15 patients, matched-unrelated donor; 5 patients, alternative donor were used. Treatment-related mortality of HSCT was observed in five (17.9%) patients. Fourteen (16.7%) among the study patients relapsed. The median survival (OS) of total 84 study patients was 21 months. Patients with intermediate risk karyotype showed better median OS than those with poor risk karyotype (22 months vs 7 months, P=0.020). The median OS was similar in patients with good and intermediate IPSS, but significantly worse in patients with poor IPSS (21 months, 7 months, 7 months, respectively, P=0.026) (Figure 1).

Figure 1.

PB1685

PREGNANCY ACCUMULATES UNFAVORABLE MOLECULAR GENETIC AML AND SHOULD BE CONSIDERED AS A POOR PROGNOSTIC FACTOR

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Background: Acute myeloid leukemia (AML) during pregnancy – is a rare clin-
including clofarabine at 22.5 mg/m² daily on days 1-5, followed after three
Days of infective disease, in this cohort of patients we did not observe a significant delay in bone
response (6%), 38/67 had resistant disease (56.7%), 3/67 died of complications
Aims: To assess the pregnancy, as independent prognostic factor, in non APL
AML-patients (pts), prospectively treated within Russian AML multicenter studies.
Methods: From 1990 to 2017 yearly the Russian Acute Leukemia study group has treated 33 with de novo AML pregant women (Me=27 (21-42) yrs), AML was diagnosed in the 1st trimester in 1 woman (3%), in the IIInd (15,45%), in the IIIInd (17 (51,5%), Molecular genetic risk group was estimated in 27/33 pts: 52% (n=14) were referred to the intermediate risk group and 48% (n=13) to the poor prognosis. High risk group comprised complex karyotype (n=9), -7/-7diploidy, translocations inv(11)q(22) (n=2), 1 pt - inv(3)/7 and 1 pt - AML with myelodysplasia-related changes, normal karyotype and FLT3-ITD.
In 1 pt at the 1st trimester medical abortion was conducted and 11 women delivered at the gestation age of 34-40 weeks before chemotherapy (CT). 21 pregnant women received CT, that started at 23 (13-32nd) weeks of gestation. Classical salvage therapy was applied in all of pts: either with daunorubicin (45-60 mg/m²), or mitoxantrone (10 mg/m²), or idarubicin (12 mg/m²) regarding the following treatment-study protocol.
Results: As our data show, AML in pregnancy is characterized by high prevalence of unfavorable cytogenetic abnormalities (48%), that is substantially different from non-pregnant women of the same age (11,5%) (p=0,006) [Blood 2016,128;22,p.5171]. 1 pt died before CT due to septic shock, 2 pts – in induction CT now. 2 pregnant women died due to severe infections in aplasia during induction (5,7%). So, induction results were evaluated in 30/33 pts: CR rate - 73,3% (22/30): after the 1st course CT - in 16 and after the 2nd - in 6 pts. In pts, with available cytogenetic data, CR was received in 100% (9/9) from the intermediate and in 80,0% (8/10) from the poor prognostic group. Primary resistance was registered in 6/30 pts (20%). Antenatal fetal mortality was registered in 2 cases at the 21st and 32nd weeks during induction. 29 children were born. Allogenic bone marrow transplantation (allo-BMT) was done in 10 of 28 (35,7%) AML-pts who had survived induction therapy at a median of 6 months after CR. 4 pts relapsed after allo-BMT and 1 woman remained with refractory AML after allo-BMT. Our results demonstrated rather low 10-y OS and DFS (10,48% and 10,46%) in women, whom AML was diagnosed during pregnancy. In order to evaluate the role of allo-BMT, we performed a landmark analysis (landmark=6 months of CR), that has shown better OS and DFS only in pts after allo-BMT (Pic 1).

Figure 1.

Summary/Conclusions: Our results demonstrate: almost half of women, who were AML diagnosed during pregnancy, are referred to the poor molecular genetic prognostic group; they demonstrated very low OS and DFS whith their improvement after allo-BMT.

PB1686
CLOFARABINE IN RELAPSED-REFRACTORY ACUTE MYELOGENOUS LEUKAEMIA: A SINGLE CENTRE EXPERIENCE

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Background: Clofarabine has been shown to be effective in AML patients, mainly in the induction treatment at high dose cytarabine.

Aims: On the basis of these reports, we tested clofarabine in association with high dose cytarabine in relapsed/refractory AML patients, selecting cases of primary refractoriness to at least two induction therapies, relapsed but refractory to a standard re-induction treatment, or very early relapse.

Methods: From 2009 to 2016 we treated 67 patients with a regimen including clofarabine at 22.5 mg/m² daily on days 1-5, followed after three hours by cytarabine at 1 gr/m² daily on days 1-5. Among the 67 patients, 24 were in first relapse, 29 in second or third relapse, 14 with resistant disease. The mean age was 54 years (range: 36-77 years).

Results: 20/67 patients achieved a complete remission (29.9%), 4/67 a partial response (6%), 38/67 had resistant disease (56,7%), 3/67 died of complications during the aplastic phase (4,4%). The most frequent non haematologic adverse events were: transient liver toxicity (41 grade 1-2, 11 grade 3-4) skin rash (33%), vomiting (28%), diarrhea (15%). Comparing with other salvage strategies, major differences were a significant delay in bone marrow recovery (median time to ANC recovery 21 days), Febrile neutropenia was observed in 58 cases (85%), with bacterial infections microbiologically documented in 20 patients (29%) and 2 cases (3%) of fungal infections. The median overall survival of the whole cohort was 115 days, with a median event free survival of 111 days. Among the responding patients, 16 (24%), underwent allogeneic bone marrow transplantation; in these selected patients, median overall survival was 185 days.

Summary/Conclusions: These results suggest that the clofarabine-ARA-C regimen was able to induce a response in about one third of this particularly poor prognostic category of patients, with a safety data consistent with previously reported salvage therapies. Nevertheless, long term results remain still and completely unsatisfactory. Further studies, with different combinations or in more selecting conditions, are warranted.

PB1687
PRESENCE OF MULTIPLE DRIVERS IN THE SELECTION OF HIGH AND LOW INTENSITY CHEMOTHERAPY IN AML

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Background: Data on the key drivers of initial treatment choice for patients diagnosed with acute myeloid leukemia (AML) in the United States is limited. The use of age as a selection driver of induction therapy is well established; however, there is limited data and a knowledge gap about additional factors driving treatment selection.

Aims: This analysis explored the key physician drivers, which led to the selection of high- and low-intensity induction therapy in AML patients.

Methods: Data from the Adelphi AML Disease Specific Programme, a real-world, cross-sectional survey conducted between February–May 2015, was analysed. A total of 61 hematologists/oncologists provided attitudinal information about their management and treatment choices for AML patients via survey. Each physician was provided a pre-specified list of 16 patient characteristics. Via two separate questions, they were asked to select those considered important when choosing high and low intensity chemotherapy for their AML patients. Characteristics were analysed descriptively and ranked based on the frequency of mention from highest to lowest.

Results: The top three drivers for decision making when selecting high and low intensity treatment were: patient age, performance status and presence of comorbidities. More than 60% of physicians would prescribe high-intensity treatment to patients aged <65, with a good performance status or with no comorbid conditions. Over half of physicians would consider those who are eligible for a stem cell transplant or have a mutation in the CEBPA gene to be eligible for high-intensity chemotherapy (Table 1). Low-intensity chemotherapy was considered by more than 60% of physicians as being the most appropriate treatment for patients aged ≥65, with a poor performance status or increased number of comorbid conditions. A total of 38% of physicians would likely consider low-intensity chemotherapy if the patient was ineligible for a stem cell transplant or had had previous cancers or exposure to radiation/chemotherapy in the past.

Table 1. Top 5 patient characteristics considered by physicians when choosing high- or low-intensity treatment in AML.
Background: IRAIN which is produced from the insulin-like growth factor type I receptor (IGF1R) imprinted locus is a newly identified IncRNA. There are very little knowledge about the specific role of this IncRNA in tumorgenesis presses. Recent studies were revealed that IRAIN is down-regulated in leukemia cell lines and viral expression of the IRAIN IncRNA inhibits tumor cell mitosis, suggesting a tumor suppressor function for this transcript.

Aims: In this study, we attempted to examine the expression level of IRAIN in different cytogenetic subtypes of AML patients.

Methods: Using quantitative polymerase chain reaction (qPCR) the expression level of IRAIN were analyzed in bone marrow specimen of AML patients (n=76) and healthy individuals (n=18).

Results: IRAIN expression in the AML was found to be remarkably decreased in AML patients compared with healthy individuals (p= 0.02). Significant IRAIN down-regulation was observed in all FAB types except for the M3 (p = 0.11). When we analyzed the expression level of IRAIN in different cytogenetic subtypes of AML patients the statistically down-regulation of IRAIN was observed only in poor prognosis groups (p<0.05).

Summary/Conclusions: Our results suggest that down-regulation of IRAIN IncRNA might play a role in the AML development and hence may be a potential prognostic factor and serve as therapeutic target for AML treatment.

PB1689

PERFORMANCE OF THE LEUKOSTRAT® CDX FLT3 MUTATION SIGNAL RATIO ASSAY TO DETECT INTERNAL TANDEM DUPLICATION AND TYROSINE KINASE DOMAIN MUTATIONS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) in general has a poor prognosis. Assessment of the mutation status of the FLT3 (fms related tyrosine kinase 3) receptor gene in AML is the most important prognostic indicator of disease outcome, which is often substantial, as many studies in AML have shown that the presence of FLT3 activating mutations portends a poor prognosis. The LeukoS-trat® CDX FLT3 Mutation Assay targets regions of the FLT3 gene to identify internal tandem duplication (ITD) mutations and tyrosine kinase domain (TKD) mutations. Since this assay is a signal ratio (SR) assay with a validated cutoff of 0.05, demonstration of international harmonization of results is paramount. FLT3 ITD mutations are caused by duplication and insertion of a portion of the FLT3 gene that includes the region in and around the juxtamembrane region of the FLT3 gene. These mutations vary in both the location and the length of the inserted duplicated DNA sequence. ITD mutations result in constitutive activation of the FLT3 kinase. FLT3 TKD mutations are caused by nucleic acid substitutions and/or deletions that result in a change in the amino acid sequence in this highly-conserved catalytic center. TKD mutations, such as D835 and I836 substitutions and deletions, result in constitutive autophosphorylation and activation of FLT3.

Aims: To assess the performance of the Invivoscribe® LeukoStrat® CDX FLT3 Mutation Assay.

Methods: White blood cells were removed from peripheral blood after 30 minutes of centrifugation at 2000 x g to create leukocyte depleted blood (LDB). Various ratios of four ITD positive cell line, with insert sizes from 21 bp to 279 bp, and one TKD positive cell line, with a D835 substitution mutation, were created over a wide range of signal ratios (0.02 to 1.83) and added to the LDB. Mononuclear cells were isolated from the contrived LDB samples. DNA was extracted and amplified via PCR. The amplicons were analyzed via capillary electrophoresis. The assay measured the ratio of signals from mutation against a background of a wild-type cell line. A FLT3 mutation was detected (and reported as positive) if the mutant/WT type ratio was over or exceeded the clinical cut-off of 0.05. Proprietary software calculated the SR and reported positive or negative. Clinical specimens were de-identified by LabPMM in San Diego. DNA from twenty specimens was tested by three laboratories: LabPMM LLC in San Diego, LabPMM GmbH in Germany and LabPMM Gk in Japan.

Results: The analytical performance of the LeukoStrat® CDX FLT3 Mutation Assay was evaluated using contrived LDB samples, with known FLT3 mutations. For limit of blank (LoB), the SR was 0.00 in the ITD assay and 0.00 to 0.01 in the TKD assay, which is well below the clinical cut-off SR of 0.05. The limit of detection (LoD) in the ITD assay detected allelic ratios of 0.03, 0.05, and 0.53 above the LoB SR in more than 95% of samples for insertions sized at 30 bp, 126 bp and 279 bp, respectively. The limit of detection in the TKD assay detected an allelic ratio of 0.05 above the LoB. For precision and reproducibility, the SR%CV was within 3-14% across ITD and TKD mutation types regardless of reagent lots, equipment or operator. There was 100% agreement between all three clinical LabPMM laboratory sites.

Summary/Conclusions: This robust assay produced a SR%CV less than 15% regardless of reagent lot, equipment or operator. The high reproducibility between the three laboratories on three different continents provides evidence that the Invivoscribe® LeukoStrat® CDX FLT3 Mutation Assay is an internationally standardized assay.

PB1690

CLINICAL FEATURES AND OUTCOME OF PATIENTS WITH CORE BINDING FACTOR ACUTE MYELOID LEUKEMIA

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Background: Acute Myeloid leukemia is classified into different prognostic groups according to their cytogenetic profile; AML with t(8;21) or inv(16) (1;16) called AML CBF belong to a prognostic group of low-risk; they represent 15% to 20% of the AML.

Aims: The aim of this study is to present clinical, cytogenetic features and outcome of this group of patients (pts) in an emerging country.

Methods: Cytologic diagnosis of AML CBF is completed by immunophenotypic and cytogenetic analysis:
t(8;21), inversion 16(t;16) and del16q22. Induction treatment: Daunorubicin 45 to 90 mg/m² day (d1-d3)+Cytarabine 100 mg/m² (d1 - d7) (with progressive doses if major leukocytosis). Assessment between d 21 and d 28 by bone marrow analysis; if failure a study. The CR rate is modestly affected by Cytarabine high dose 3 g m²/12 h d1, d3, d5. Central nervous system prophylaxis for patients (pts) with AML-M5 and hyperleukocytosis forms. Consolidation: Cytarabine high dose: 2 to 3 cures; low dose of chemotherapy for pts older than 55 years or pts presented severe toxicity at the first induction. Research of FLT3 and residual disease is not available in our laboratory; hematological stem cell transplantation (HSCT) was proposed for all pts; if no compatible donor, 3 courses of Cytarabine high doses was instituted.

Results: From 2010 to 2016, cytogenetic analysis was performed in all cases of AML of which 58 cases(18,6%) of LAM - CBF were diagnosed; the ratio to female ratio was 0.5 ; average age: 37 years (16-72); t (8;21) was found in 28 pts (16 M,12F); inversion (16)(p13.11;q22.1), t(16;16)(p13.11;q22.1) and del16q22 were found in 30 pts (12M,18F), respectively in 27 pts, 2 pts and 1 pt. Four cases of del(16)(p13) were associated with inv(16). For inv(16), FAB sub-types were AML4 (26), AML2 (1) and 3 AML; For t(8;21), there was 26 AML2 and 2 AML4. Evaluation of induction: not evaluable: 13 cases, Complete Remission (CR): 38 cases (65,5%); for 7 cases in failure , a second induction was proposed, we obtained 2 CR. 15 pts were transplanted. Outcome: 27 pts are alive in CR of which 12 transplanted . 31 pts died of which 18 toxic deaths ( 15 after induction treatment and 3 after engraftment). Median overall survival for inv(16): 11 months vs 15 months for t(8;21) (p=0.87).

Summary/Conclusions: In our study, the frequency of the CBF AML is closer than those described in another Algerian study and literature: 18,6% vs 15.4% and 20% respectively; a slight predominance of the inv 16 or t (16;16) identical to that of the CALGB study. We noted less relapse compared with literature. Relapses were observed in pts with poor prognostic factors: age, leukocytosis and failure to first induction. Regarding the favorable prognosis of AML CBF, our results are bad because the high rate of toxic deaths. The CBF AML are characterized by a good induction diagnosis, but with a 30% relapse rate essentially when associate poor prognostic factors such as a kit mutation that increases the risk to 70%, mutation FLT3, advanced age, the leukocytosis, severe thrombocytopenia and additional cytogenetic abnormalities.

PB1691

FLOW CYTOMETRY ANALYSIS SOFTWARE FOR REMOTELY LOCATED HAEMATOLOGISTS

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Background: Current flow cytometry software packages are unsuitable in cases where the interpreter of the data isn’t physically located at the computer with the software installed. This is particularly disadvantageous in urgent situations, such as in the diagnosis of acute leukemia.

Aims: Develop a tool to allow haematologists to analyse flow cytometry data from anywhere on any internet-enabled device e.g. tablet, smartphone, laptop, PC.

Methods: We came up with principles a new software package should adhere to: 1. should be accessible from any Internet-enabled device e.g. iPad, Android phone, Blinders/phone; 2. should not require installation; 3. FCS data should be anonymised; 4. data transfer should be secure and encrypted; 5. software must include all basic functionality of flow cytometry software e.g. dot plot graphs, histogram graphs and gating 6. should put collaboration to the forefront e.g. analysis can be instantly linked to via a web URL.

Results: The resulting software package is a web app which is accessible from any Internet-enabled device such as an iPad, touch is used for drawing of gates, selection of quadrants, selections of parameters etc. on laptop’s and PCs, these are drawn via
Background: A variety of different treatment regimens have been studied in an effort to improve outcomes of patients with relapsed or refractory acute myeloid leukemia (RR-AML), there appears to be no single superior approach. Spanish hematologists, who received intensive induction chemotherapy with FLAG-IDA protocol at our hospital between January 2007 and December 2016. Biodemographic, clinical and pathological variables were recorded. We analyzed the response rate, the 30-day mortality rate and overall survival that other groups in our country. Despite a variety of salvage therapy options, like FLAG-IDA protocol, prognosis in patients with RR-AML is generally poor and treatment is very complex.

Methods: Descriptive study of a case series of patients with acute leukemia who received intensive induction chemotherapy with FLAG-IDA protocol at our hospital between January 2007 and December 2016. Biodemographic, histopathological, cytogenetic and molecular results and previous treatment were recorded. We analyzed the response rate, the 30-day mortality rate and the overall survival.

Results: 65 patients received treatment with FLAG-IDA protocol between 2007-2016, 36 of them female, with and average age of 53.4 years (DS+/-23.3). We treated with this protocol mostly patients with relapsed or refractory acute myeloid leukemia (RR-AML) (primary refractory or resistant AML as defined by not achieving complete remission after 1 cycle of intense induction therapy); 60% (n=38) of patients had a RR-AML, 37% (n=23) of them were relapsed AML (defined as 23% blast per bone marrow aspirate [BM]/[20% per BM]) and 6% (n=4) refractory AML. Based on European Prognostic Index Score (EPI-SCORE) for patients with RR-AML, 61% of them had a poor prognosis (10-14 points), 36% had an intermediate prognosis (7-9 points) and only 3% had a favorable prognosis (1-6 points). The next important group, 25% (n=17) were MDS patients transformed to AML. We had 9% (n=6) patients with treatment related AML and 6% with other acute leukemia (3 cases of refractory ALL and 1 case of biphenotypic leukemia). We observed a global response rate of 63%; 51% (n=33) of patients had a complete response (CR) and 12% (n=8) partial response, 17% (n=11) did not have a response and 20% of patients were not evaluated after to receive the treatment because they had a early death. The 30-days mortality rate was 21.5% (n=14), similar to the unmeasured patients. We can see in the overall survival curve (picture 1) that most patients died first months after treatment, after that patients remain alive and we achieve a plateau. The median overall survival was 82 days (standard deviation: 25 days): 10 patients were alive when we analyzed the data (Figure 1).

Summary/Conclusions: Most AML patients ultimately die from their disease. In our case serie none died by any other cause. We had a similar response rate, mortality and overall survival that other groups in our country. Despite a variety of salvage therapy options, like FLAG-IDA protocol, prognosis in patients with RR-AML is generally poor and treatment is very complex.

PB1693
BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASMS - UNUSUAL PRESENTATIONS AND UNFAVOURABLE OUTCOMES
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Background: Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare hematological malignancy with an aggressive clinical course. Most patients (pts) with BPDCN have skin lesions and involvement of peripheral blood, bone marrow, and lymph nodes. Very few cases have been described with lack of skin and or bone marrow manifestations at the time of diagnosis.

Aims: To characterise the clinical presentation and clinical outcomes of a cohort of consecutive patients with a rare blastic plasmacytoid dendritic cell neoplasm in a single institution.

Methods: Patients diagnosed with BPDCN at the National Hematology Hospital between 2010 and 2016 were retrieved from the database. The diagnosis was confirmed by morphology and immunophenotyping by flow cytometry and/or immunohistochemistry, according to 2008 WHO Classification of Hematopoietic Neoplasms. The relevant clinicopathologic features were reviewed.

Results: We identified 8 adult patients at a median age of 70 years (range: 37-84 years) with a male:female ratio of 6:2 (75%:25%) and only 1 child. Mean values of blood cell counts were as follows: WBC 5.10^9/L; hemoglobin 99 g/L; platelets 116.10^9/L. LDH was generally elevated with a mean of 962.8 U/L. At diagnosis the skin was involved in 7/8 patients. Five patients developed leukemic presentation with 40-95% of bone marrow infiltration. Interestingly, in 4 pts (50% of adult pts) the initial presentation affected other tissues and organs such as testis, bronchial wall, stomach and periportal soft tissues, however, only the latter one case presented with a leukemic picture. Biopsies revealed diffuse, monomorphic infiltration of medium-sized blast cells with irregular nuclei, fine chromatin with 1± nucleioli, scant and agranular cytoplasm, without angioinvasion or coagulation necrosis. Immunophenotype generally demonstrated CD45+, CD4+, CD56+, CD123+. No standard therapies were applied. Patients received CHOP or HyperCVAD or AML-induction therapy. However, response rates in adult patients were low and the mean OS was 2.6 months (ranging from early deaths before any treatment could be initiated to 10 months).

Summary/Conclusions: BPDCN is a rare aggressive disease that typically affects elderly patients. The most commonly affected non-hematopoietic organ is the skin, however any other organ or tissues can also be involved. Response to therapy if any is relatively short and long-term prognosis is poor despite of the site of presentation. Larger scale studies are warranted to understand the pathophysiology of the disease and to find optimal management.

Acknowledgements: Partial support by the National Science Fund.
Background: Western hospitals have achieved First Complete Remission (CR-1) and Overall Survival (OS) rates of 90% and 60% for children with Acute Myeloid Leukemia (AML). Intensified regimen of standard chemotherapy along with precise risk classification and improvements in supportive care are mainly attributed to this achievement.

Aims: We analyzed clinical data of our pediatric AML patients treated at KFSH&RC from 2005 to 2015 in order to assess the outcome of our treatment efforts including Hematopoietic Stem Cell Transplantation (HSCT).

Methods: A total of 155 pediatric patients with AML were registered at our institution from 2005 to 2015. 55.5% (86) were boys with a F:M ratio of 1:1.2. The aim of the study was to determine the role of HIF-2 alpha in human AML.

Results: Our CR-1 rate was 93.7% (134 of 143) with 100% in Low Risk, 95.2% Intermediate Risk and 85.7% in High Risk patients (P-Value: 0.023), requiring 1-3 cycles of chemotherapy with a median time of 1.3 months. Treatment Failure was observed in 6.3% (9 of 143). Relapse rates were 38.8% (52 of 134). Most common site of relapse was bone marrow (75%, 39 of 52) followed by (22%) 11 of 52) had PML/RAR (+). Trisomy 4, 10 or 17 was not seen among any of these cases analyzed. Most commonly observed FAB classification was M-5 (23.5%, 24 of 102) followed by M-2 (18.6%). 27.3% (39) were Low Risk, 43.4% (62) Intermediate and 29.4% (42) High Risk. 43.3% (58 of 134) received HSCT.

Results: In all samples leukemic blasts were counted and determined by flow cytometry and the subpopulation of HIF-2 alpha positive blasts was estimated as well. Volunteer bone marrow donors were the control group in this study and the CD34+HIF-2α+ subpopulation was assessed in their bone marrow samples during the routine harvest procedure. The study was approved by the local Ethics Committee.

Results: After the first line chemotherapy 15 patients achieved complete remission (CR-group) and 11 did not (NR group). We did not find significant differences between the groups regarding patients age, the mean percentage of blasts in bone marrow and blood before the treatment, the percentage of HIF-2 alpha positive blasts in BM and blood before and after 48 hours after the treatment start (data not shown). But the analysis of the percentage of HIF-2 alpha positive blasts in blood before and 48 hours separately in CR and NR groups revealed quite different dynamics. In CR group the mean percentage of HIF-2 alpha positive blasts was 14.65 (±33.32) and 8.48 (±11.63) before and after chemotherapy respectively (p=NS); in NR group the values were 11.74 (±22.6) and 24.01 (±33.68) respectively (p<0.007) (Figure 1). The Cox analysis revealed HIF-2 alpha positive blasts in blood after chemotherapy to be proportional to death probability (p=0.0036) (Figure 2).

Summary/Conclusions: We are aware our results are preliminary. But if they are confirmed it will be very interesting to determine the role of HIF-2 alpha inhibitors in improving the prognosis and survival in human AML.

PB1695
IS HIF-2 ALPHA A POOR PROGNOSIS FACTOR IN HUMAN ACUTE MYELOID LEUKEMIA? A SINGLE CENTER ANALYSIS - PRELIMINARY RESULTS

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Background: Hypoxia-inducible transcription factors (HIF) are well known regulators of cellular response to hypoxia. HIFs control functional, metabolic and vascular adaptation to hypoxia on transcriptional level. HIF-1 alpha has been synthesized from 1 μg of total RNA. Most common AML genetic alterations described in acute myeloid leukemia (AML) are known. The results of our treatment efforts are in conformity with the western literature. Precise risk classification can be a vital predictor in planning for first line and salvage therapies including HSCT for pediatric patients with AML.

Methods: We analyzed a 26 primary AML patients group (median age 54.5 (21-77), F/M – 13/13). The group consisted of 21 AML-NOS cases, 2 AML cases with inv(16), one case with t(6;9) and one with t(9;11) according WHO classification. ELN cytogenetic risk stratification divided the group into intermediate-1, intermediate-2 and adverse cases in 10, 12 and 4 patients respectively. All patients were treated with Daunorubicine, Cytarabine and Cladribine based first line chemotherapy. We collect bone marrow and blood samples before chemotherapy and blood samples alone 48 hours after chemotherapy start. In all samples leukemic blasts were counted and determined by flow cytometry and the subpopulation of HIF-2 alpha positive blasts was estimated as well. Volunteer bone marrow donors were the control group in this study and the CD34+HIF-2α+ subpopulation was assessed in their bone marrow samples during the routine harvest procedure. The study was approved by the local Ethics Committee.

Results: After the first line chemotherapy 15 patients achieved complete remission (CR-group) and 11 did not (NR group). We did not find significant differences between the groups regarding patients age, the mean percentage of blasts in bone marrow and blood before the treatment, the percentage of HIF-2 alpha positive blasts in BM and blood before and after 48 hours after the treatment start (data not shown). But the analysis of the percentage of HIF-2 alpha positive blasts in blood before and 48 hours separately in CR and NR groups revealed quite different dynamics. In CR group the mean percentage of HIF-2 alpha positive blasts was 14.65 (±33.32) and 8.48 (±11.63) before and after chemotherapy respectively (p=NS); in NR group the values were 11.74 (±22.6) and 24.01 (±33.68) respectively (p<0.007) (Figure 1). The Cox analysis revealed HIF-2 alpha positive blasts in blood after chemotherapy to be proportional to death probability (p=0.0036) (Figure 2).

Summary/Conclusions: We are aware our results are preliminary. But if they are confirmed it will be very interesting to determine the role of HIF-2 alpha inhibitors in improving the prognosis and survival in human AML.

PB1696
RARE BCR/ABL FUSION PROTEINS AND THEIR CLINICAL SIGNIFICANCE INTO PH+ ACUTE MYELOID LEUKEMIAS

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Background: The Philadelphia (Ph) (t(9;22)(q34;q11) results in an oncogenic BCR/ABL gene fusion, representing the hallmark of chronic myeloid leukemia (CML), although it has been also described in acute lymphoblastic (ALL) and myeloid (AML) leukemia. Three main different transcripts have been described (p210, p190 and p230), but rare atypical BCR breakpoints outside the cluster regions have been also reported and their clinical significance is under investigation. Atypical transcript p190 e6a2 is a rare fusion protein associated with aggressive phenotype and dismal prognosis. The breakpoint in BCR intron 6 is responsible for increased kinase activity and greater transforming potential because of the partial loss of the Guanine Exchange Factor (GEF)/diBLike domain, completely absent in p190 proteins. This truncation could increase the BCR/ABL oncogenic activity.

Aims: In this report we describe 2 rare cases of Ph+ AML patients with the atypical p190 e6a2 isoform.

Methods: Routine morphologic, immunophenotypic and genetic analyses were carried out in all samples at diagnosis. cDNA extracted from bone marrow was synthesized from 1 μg of total RNA. Most common AML genetic alterations were investigated and a quantitative RT-PCR (qRT-PCR) for p190 transcripts was performed. qRT-PCR assay for FLT3-ITD and p190 e6a2 transcript were recently described for clinical use.

Results: Case 1. A 78-years old male was admitted at our hospital with clinical and laboratory features allowing to make the diagnosis of AML. No evidence of a preceding CML (spleenomegaly or basophilia) was found. The karyotype analysis was ongoing, the patient started treatment based on decitabine. The molecular biology analysis revealed the simultaneous presence of the common p190 e1a2 and the rare e6a2 isoforms (Figure 1A). Because of persisted pan cytopenia and presence of blasts, according to the molecular data, he was then switched to TKIs treatment. Nevertheless, after 2 months, the patient was still off chemotherapy due to bleeding complications. Case 2. A 61-years old male was admitted with a diagnosis of AML 46XY, FLT3-ITD post MDS. Immediately, after cytoreduction, chemotherapy was started, obtaining the complete remission. Because of infectious complications, the
consolidation chemotherapy was postponed, relapsing without reach the already planned transplantation. At the bone karyotype was 46XY, t(9;22)(q34;q11) and the molecular biology showed the presence of p190 e1a2 and e6a2 isoforms and FLT3-ITD mutations with a low mutant allelic burden (Figure 1B). Salvage chemotherapy was then performed, allowing at this time to obtain disease remission and further allogeneic transplantation. Nevertheless, the patient died 5 months later for transplant complications. qRT-PCR assays performed in diagnosis sample showed the main clone FLT3-ITD accompanied by subclones with p190 e1a2 and e6a2 isoforms. These data indicate a clonal selection process and the expansion of a resistant clone with their OS was dismal. So HMA could be a good clinical option in a selected population of relapsed patients, especially in those not suitable for allogeneic bone marrow transplantation, in whom the prognosis is generally extremely poor. Further studies are needed to determine which are the cytogenetic subsets of patients who could benefit from such a salvage chemotherapy.

**Summary/Conclusions:** The atypical p190 e6a2 transcript seems to be associated in AML with aggressive disease. TKI therapy alone does not seem to control the disease. Prompt observations on these patients carrying rare BCR/ABL transcripts may allow help to establish optimal treatment approaches on these aggressive BCR/ABL phenotypes.

**PB1697**

**HYMOPETILATING AGENTS AS SALVAGE THERAPY IN RELAPSED OR REFRAC'TORY AML: A 2-CENTERS RETROSPECTIVE STUDY**

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**Background:** 5-azacytidine and decitabine have been widely studied as first line chemotherapy in acute myeloid leukemia (AML) patients not eligible for allogeneic stem cell transplantation but data on their use as salvage chemotherapy are limited.

**Aims:** To define efficacy and feasibility of hypomethylating agents (HMA) as salvage chemotherapy in patients without previous allogeneic stem cell transplantation.

**Methods:** We retrospectively reviewed clinical records of 15 patients treated with HMA as salvage therapy in our institutions since their introduction in clinical practice for AML patients.

**Results:** Median age was 66 years. Six patients were men and 9 women. One patient was AML with t(18;21), 7 were AML MRC. 1 was therapy-related AML. 6 were AML NOS. Two patients were favorable risk sec ELN 2010, 11 were inter- medium I and II and 2 were adverse risk. 67% of patients received HMA as second line therapy for their disease, 27% as third line and 6% were beyond the number of HMA cycles was 2 (range 1-31). 26% of patients underwent intensive chemotherapy (i.e. FLA like or 3+7 like) as first line induction, and we excluded patients who had a HMA as first line chemotherapy and another one as second line. Median number of hospitalization days during HMA therapy was 26; median number of HMA cycles was 2 (range 1-31). 28% of patients underwent allogeneic stem cell transplantation after HMA therapy. Median OS was 197 days from the starting of HMA and median EFS was 70 days. Median OS in patients with refractory disease was 91 days and median OS in patients with relapsed disease was 331 days (p=0.0049). Median EFS in patients with refractory disease was 75 days and median EFS in patients with relapsed disease was 196 days (p=0.039). We did not find significant differences between transfusion needs before and after sal- vage therapy but this could be due to the small size of our sample.

**Summary/Conclusions:** HMA showed efficacy and a considerable OS in our patients. In our cohort refractory patients were almost all refractory to HMA too, and their OS was dismal. So HMA could be a good clinical option in a selected population of relapsed patients, especially in those not suitable for allogeneic bone marrow transplantation, in whom the prognosis is generally extremely poor. Further studies are needed to determine which are the cytogenetic subsets of patients who could benefit from such a salvage chemotherapy.

**PB1698**

**OMITTING CYTARABINE FROM CHEMOTHERAPY FOR ACUTE PROMYELOCYTIC LEUKEMIA REDUCES TOXICITY AND NOT EFFICACY**

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**Background:** The introduction of retinoic acid (ATRA) has changed the treatment paradigm in Acute Promyelocytic Leukemia (APL). Combination of ATRA plus anthracycline-based chemotherapy has shown high efficacy in Spanish and Italian studies. However, early mortality resulting from coagulation disorders remains high. Furthermore, AraC administration during consolidation is questioned and often limited to high-risk patients.

**Aims:** We aim to compare the efficacy, tolerance and toxicity between 2 consecutive treatment protocols that differed in AraC administration during consolidation.

**Methods:** We studied clinical characteristics, prognostic factors, response to treatment, toxicity, and outcomes in APL patients treated in our Department during the last decade. All patients received induction with AIDA (Idarubicin x4, ATRA until remission) and 2-year maintenance therapy. Protocol 1 included 2 cycles of consolidation with anthracyclines/AraC. Protocol 2 was implemented the last 5 years and included 3 cycles of anthracyclines and AraC only in high-risk patients (PETHEMA LPA2005).

**Results:** APL was diagnosed in 35 patients, of whom 2 patients older than 80 years did not receive treatment and were not included in the analysis. The rest 18 male: 15 female patients aged 37-70 years old presented at diagnosis with: thrombocytopenia (32), leukopenia (22), leukocytosis (6), impaired performance status/PS >2 (10), lactate dehydrogenase >400 IU (17), increased d-dimers (33), low fibrinogen (11), fibrinogen <1 mg/dl (5). Five patients died during induction from severe differentiation syndrome (2), bleeding (2) and infection (1). In the multivariate analysis, these patients had significantly impaired PS (3, p=0.005), older age (median of 59 years, p=0.014) and lower fibrinogen (median of 0.9 mg/dl, p=0.05). Among 28 patients eligible for the comparison, all patients achieved complete remission (CR/CRi 100%). PFS was 197 days from the starting of HMA and median EFS was 70 days. Median OS in patients with refractory disease was 91 days and median OS in relapsed patients was 331 days (p=0.0049). Median EFS in patients with refractory disease was 75 days and median EFS in patients with relapsed disease was 196 days (p=0.039). We did not find significant differences between transfusion needs before and after salvage therapy but this could be due to the small size of our sample.

**Summary/Conclusions:** The atypical p190 e6a2 transcript seems to be associated in AML with aggressive disease. TKI therapy alone does not seem to control the disease. Prompt observations on these patients carrying rare BCR/ABL transcripts may allow help to establish optimal treatment approaches on these aggressive BCR/ABL phenotypes.
initial AML diagnosis including symptoms, performance status, and physician-determined prognostic category were taken from physician-completed patient record forms. Details about subsequently prescribed AML treatment were also taken from this data source. Treatments for n=15 (3.3%) patients were reasigned as high or low intensity following evaluation of physician treatment selection. Post-hoc T-tests and Chi-Squared/Fisher’s exact tests were used to determine differences between groups.

Results: Table 1 shows key presenting characteristics of AML patients <60 and ≥60 years of age. According to physicians, those patients <60 years of age were significantly more likely than those ≥60 years of age to have de novo AML, a performance score of 0 versus ≤1 at diagnosis, more tests conducted to establish the diagnosis and a more favorable prognosis at baseline, according to physician perception. Following initial diagnosis, patients <60 years of age were 1.65 times more likely than patients ≥60 years of age to be initiated on high-intensity induction treatment: 67% (n=143) of patients <60 years of age compared to 40% (n=98) of patients ≥60 years of age (high versus low intensity by age group P < 0.001). All other patients received low intensity treatment. Irrespective of age, the most common high intensity treatment given was a cytarabine-based regimen and the most common low intensity treatments were low dose cytarabine-, decitabine- or azacitidine-based regimens.

Table 1. Disease characteristics of patients <60 and ≥60 years of age at diagnosis of AML.

<table>
<thead>
<tr>
<th>Disease characteristics</th>
<th>&lt;60 years old (n=244)</th>
<th>≥60 years old (n=263)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td></td>
<td>129 (53%)</td>
<td>95 (36%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17 (7%)</td>
<td>14 (5%)</td>
<td></td>
</tr>
<tr>
<td>Pathophysiology</td>
<td>Anemia</td>
<td>Leukocytosis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>208 (86%)</td>
<td>209 (84%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>4 (2%)</td>
<td>5 (2%)</td>
<td></td>
</tr>
<tr>
<td>Symptoms</td>
<td>No. of symptoms at diagnosis (mean [SD])</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.5 (3.3)</td>
<td>5.2 (2.9)</td>
<td>0.107</td>
</tr>
<tr>
<td>Performance status</td>
<td>ECOG score at diagnosis - 0 (Fully active, able to carry on all pre- disease performance without restriction)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>41 (17%)</td>
<td>43 (18%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diagnostic tests</td>
<td>No. of tests used to establish AML diagnosis [mean [SD]]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.3 (3.6)</td>
<td>4.7 (3.6)</td>
<td>0.025</td>
</tr>
<tr>
<td>Diagnosis-defined prognostic category</td>
<td>Favorable</td>
<td>Intermediate</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>101 (41%)</td>
<td>84 (34%)</td>
<td>22 (9%)</td>
</tr>
<tr>
<td></td>
<td>24 (22%)</td>
<td>12 (15%)</td>
<td>55 (23%)</td>
</tr>
<tr>
<td>Blood cell number</td>
<td>10魅力</td>
<td>9魅力</td>
<td>8魅力</td>
</tr>
</tbody>
</table>

Summary/Conclusions: The age of an AML patient at initial diagnosis appeared to play a significant role in the diagnostic, prognostic and treatment intensity decisions made by AML-treating physicians in the US. The estimated performance and prognostic status tend to be considerably better for younger patients and consequently, they were more likely to receive the most aggressive yet even more effective high intensity treatments currently available to treat AML.

PB1700

FLT3, NPM1, CEBPA and TP53 MUTATIONS AT ACUTE PROMYELOCYTIC LEUKEMIA: PROGNOSTIC FACTORS AND CORRELATION WITH OTHER MARKERS WITHIN THE PATIENTS OF GOMEL REGION IN BELARUS

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Background: Acute Promyelocytic Leukemia (APL) is one of the favourable variants of acute myelogenous leukaemias due to its ability of ATRA in the treatment simulating antithrombotic therapy. But relapses occur in 13-33% cases after achievement the remission and there are cases of early death from the bleeding. High leucocytosis, the presence of lymphoid immunophenotypic markers and gene mutations are important prognostic factors.

Aims: To examine prognostic factors in APL.

Methods: The materials for research were the samples of whole venous blood and bone marrow of 40 patients with APL treated in the period of 2009-2016 in Hematology department for adults, Gomel. The diagnosis was proved by the presence (t(15;17)) or PML/RARA. Induction therapy was carried out according to the protocol <173> using ATRA. Immunophenotypic analysis was carried out by standard immunofluorescence methods. The method of polymerase chain reaction (PCR) with specific primer and following electrophoretic detection was used for recognition of gene mutations.

Results: Out of 40 examined patients (mean age 48.5, 80.32) achieved remission and 15.6% (5) subsequently relapsed after the first course of chemotherapy. Clinical, laboratory, molecular genetic and immunophenotypic data which could affect remission results and general survival rate were analyzed within all the patients. As a result, mutations were detected in 55% of cases, FLT3-ITD mutations were detected in 32.5% (13), NPM1 mutations in 12.5% (5), FLT3-ITD and NPM1 mutations in 7.5% (3), TP53 and CEBPA mutations were detected in 5% (2) and 12.5% (5) of cases respectively. After achievement of remission after the first course of chemotherapy NPM1 mutation remained at 6.2% (2). Mutations were identified more frequently within the patients with the absence of response to the therapy or with the developed relapse. Our results prove that the presence of only one of the signs is not a factor of high risk. Only combination of clinical, laboratory, molecular-genetic and immunophenotypic markers can include the patients into a high risk group and influence general survival rate.

PB1701

A UNIQUE PRESENTATION OF ACUTE PROMYELOCYTIC LEUKEMIA: AORTOILIAC OCCLUSIVE DISEASE (LERICHE SYNDROME)

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Background: Acute promyelocytic leukaemia (APL), FAB M3 subgroup of acute myeloid leukaemia is known for its association with haemostatic disorders. Compared to bleeding thrombosis is a less commonly encountered complication of APL. Thrombosis of major arteries is a rare form of presentation.

Aims: A case, who applied with acute lower limb ischemia and diagnosed with APL and aortoiliac occlusive disease (Leriche syndrome), is presented.

Methods: A 53-year-old female patient presented with weakness, loss of appetite and pain in the lower extremities. She had diabetes mellitus (DM) regulated with metformin, hyperlipidemia (HL), and smoking history. Physical examination revealed general paleness and ischemia around big toe of the right foot. Laboratory studies revealed leukopenia, neutropenia, anaemia, thrombocytopenia, elevated D-Dimer. A bone marrow aspiration and biopsy was done to enlighten the etiology of pancytopenia. The pathological examination of the bone marrow revealed abundant granular blasts (78%) and Auer rods. The patient was diagnosed with APL, hypergranular classical form. t(15;17) was positive with fluorescence in situ hybridization. All-trans retinoic acid (ATRA) plus idarubicin treatment was started. In few days symptoms of ischemia progressed and encompassed 2nd, 4th and 5th toes together with the big toe (Figure 1 on the right). Low-molecular-weight heparin therapy was started. According to rheumatological tests and tests for lupus anticoagulant, anticardiolipin and antiphospholipid antibodies, anti-beta-2 glycoprotein 1, protein C-S, Antithrombin III and homocysteine levels, methylenetetrahydrofolate reductase, Factor V Leiden and prothrombin gene mutations no cause of tendency to thrombophilia could be determined. Echocardiography was normal.

The patient was transferred to Cardiovascular Surgery Department for axillofemoral bypass operation.
which makes our case unique. Thrombotic risk factors in APL include high leukocyte count, presence of coagulation disorder, ATRA+chemotherapy+ antifibrinolytic therapy and ATRA syndrome. None of these were seen in the presented case. The effects of known predisposing risk factors to thrombosis meaning DM, HL and smoking cannot be ruled out. But development of acute thrombosis concomitant with APL diagnosis points out to the relationship between these two entities.

Summary/Conclusions: Current literature knowledge is based on case reports and 9 patients with APL who presented with acute lower limb ischemia were reported yet. As far as we know our case is the first APL case presenting with aortoiliac occlusive disease (Leriche syndrome).

PB1702
A CASE OF THERAPY-RELATED ACUTE LEUKEMIA WITH MIXED PHENOTYPE WITH BCR-ABL1 AFTER TREATMENT OF DIFFUSE LARGE B-CELL LYMPHOMA
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Background: Although therapy-related acute leukemia (tAL) is a well-recognized clinical syndrome and is increasing owing to the prolonged survival of patients treated with chemoradiotherapy, tAL with mixed phenotype is extremely rare.

Aims: Here, we report a rare case of tAL with mixed phenotype with BCR-ABL1 after achieving complete remission (CR) of Diffuse Large B-Cell Lymphoma (DLBCL).

Methods: A 57-year-old woman was diagnosed as DLBCL. The patient received six cycles of R-CHOP regimen with G-CSF injected after each cycle and achieved CR. The patient was readmitted to the hospital after a follow-up examination revealed the presence of immature cells in the blood.

Results: Her complete blood count findings were as follows: hematocrit, 35.1%; hemoglobin, 116 g/L; platelet count, 129×10^9/L; and white blood cell count, 2.41×10^9/L, with 4% blasts, 26% segmented neutrophils, 3% band neutrophils, 39% lymphocytes, and 26% monocytes. Bone marrow aspirations revealed 40.7% blasts with medium cell size, oval-shaped, shape vesicular nuclei, fine chromatim patterns, and basophilic cytoplasm. On cytochemical staining, these blast cells were not positive on PAS and NSE staining, but were weakly positive for MPO staining. Flow cytometric analysis showed that the blasts were positive for both T-lymphoid and myeloid markers (cytoplasmic CD 3,87%; CD 5,90%; CD 7,96%; cytoplasmic myeloperoxidase, 20%; CD 13,91%; CD 33,87%) and negative for CD2, CD10, CD14, CD15, CD19, CD20, CD61, CD117, and TDT. Immunophenotyping fulfilled the diagnostic criteria of T/myeloid biphenotypic leukemia based on the scoring system of the EGIL and WHO classifications. Multiplex reverse transcription PCR using human leukemia cell lines and bone marrow aspirates revealed the presence of minor BCR-ABL1 (e1a2) fusion transcripts. Chromosome analysis of bone marrow cells failed because of insufficient mitotic cells. Immunoglobulin heavy chain gene rearrangement and TCR gene rearrangement were not detected on bone marrow aspirates.

Summary/Conclusions: Mixed phenotype acute leukemia is an uncommon subtype that comprises 0.5%-1% of leukemia. The T/myeloid phenotype is rarer and represents 35% of all MPAL cases. The risk of secondary malignancies after lymphoma treatment is relatively increased for leukemia. AML, ALL, MDS, CML and chronic myelomonocytic leukemia are reported secondary hematologic malignancies. Until now, only one case of tAL with mixed phenotype after lymphoma has been reported worldwide. To the best of our knowledge, this is the second case of tAL with mixed phenotype after DLBCL. This case is also unique because the BCR-ABL1 has not been described in the literature for patients with AML with mixed phenotype, after hematologic malignancy. According to the 2008 WHO classification, tAL can be attributed to radiation, alkylating agents, or topoisomerase II inhibitors. Our patient did not receive radiation therapy but previously received cyclophosphamide and doxorubicin. Therefore, this is the first case of tAL with mixed phenotype and BCR-ABL1 after alkylation agent and topoisomerase II inhibitor therapy for DLBCL.

PB1704
CLINICAL, CYTOMORPHOLOGIC AND IMMUNOPHENOTYPIC CHARACTERISTICS OF PATIENTS WITH BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM - DIAGNOSTIC AND THERAPEUTIC DILEMMA
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Background: Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a clinically aggressive haematological malignancy that originates from clonal proliferation of plasmacytoid dendritic cells and their precursors. BPDCN is rare, represents less than 1% of acute leukemias. The disease has two patterns of presentations: cutaneous and leukemic. The main histological differential diagnosis includes: cutaneous NK/T-cell lymphoma; cutaneous T-cell lymphoma with co-expression of CD56 and CD56+ acute myeloid leukemia with monocytic differentiation.

Aims: The aim of study was to analyze heterogeneity of BPDCN differential diagnosis, especially with regards to clinical, immunological and cytomorphological characteristics of blastoid cells in terms of the optimal treatment.

Methods: During period 2010-2016. at the Clinic of Hematology, eight patients with BPDCN were diagnosed (M/F 6/2; average age 38 yrs, range 26-60yrs).

Results: Median WBC 6,38x10^9/L (range 2,6-12); Plt 147,8x10^9/L (range 20-282). Hemor- rhagic diathesis was registered in 3/8; splenomegaly in 6/8 (average diameter by ultrasound exam 140mm, 110-150mm); and hepatomegaly existed in 3/8 (average diameter 166mm, 140-200mm); cutaneous infiltrations were pres- ent in 5/8 pts as livid maculopapular rash along lower extremities in 5 pts, and in 1 female pts in the breast region of 1-4cm diameter. In all 5 pts, immunocyto-chemistry confirmed BPDCN diagnosis. In the bone marrow aspirates of 7/8 pts, average 75% infiltration (27-89%) with blasts was revealed. Cells were of median size, with high nucleus cytoplasm ratio, with visible oval or slightly imprinted nuclei. Basic immunophenotype profile was characterised by expression of CD56+CD123+high CD45RA-, and negativity for CMPO- cCD79a- cCD3c- in 4 cases. Immunohistochecistry staining in the rest of 4 pts, characterized with dry aspiration, revealed LCA+CD5+CD64+CD123+ positivity and MPO- CD13+CD117- CD68- HLA-DR+. Cytogenetic analysis revealed normal karyotype in 4 pts, while the rest of 4 pts had pathological findings: 1. 92,XX, XY; 2. 80-120,XY,XY+ mar (16)46XY; 3. 46XY,del 5q46XY; and 4. 46XX,del 12p, respectively.

Summary/Conclusions: RT-PCR cannot substitute conventional cytogenetic diagnosis due to the absence of a broad based application for detection of aberrations other than translocations. However, given its efficiency and reliability it can have a complimentary role in prognosis assessment.
**Results:** Four pts were treated with 3+7 chemotherapy. Complete remission (CR) was achieved in 3 pts, and treatment was continued according to the HIDAC and IDAC protocol. The duration of remission was 3, 8 and 11 months respectively, followed with relapse and fatal outcome. One of the pts died within first 0.5 months after BPDCN was diagnosed. Three pts, treated with Hyper-CVAD, are alive and in CR with duration of 1, 3 and 10 months respectively. The continuation of the treatment within the programme of allogeneic stem cell transplantation is planned in 2 pts.

**Summary/Conclusions:** BPDCN diagnostics is difficult due to the heterogeneity of immunological characteristics of disease. Aggressive course of disease with median survival of 12-18 months, in the view of the unique treatment recommendations indicates necessity of further clinical investigations on larger patients groups.

**Aggressive Non-Hodgkin lymphoma - Clinical**

**PB1705**

**ECONOMIC IMPACT AND HEALTHCARE UTILIZATION IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA IN ROUTINE CLINICAL CARE IN THE UNITED STATES – A CLAIMS DATABASE STUDY**

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1Millenium Pharmaceuticals, Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited, Cambridge, 2Xcenda LLC, Palm Harbor, United States

**Background:** DLBCL is the most common histologic subtype of non-Hodgkin lymphoma (NHL), accounting for about 33% of all NHL cases. However, the healthcare burden associated with DLBCL has not been extensively studied in a US population.

**Aims:** We evaluated the costs of care and healthcare utilization (HCU) of DLBCL patients treated during routine care in the US.

**Methods:** The Optum claims database was used to identify adult patients (≥18 years old) with newly diagnosed DLBCL between 01/01/08 and 10/31/15. DLBCL diagnosis was based on ≥1 inpatient claim or ≥2 outpatient claims with DLBCL diagnosis codes, with the index date being the first DLBCL claim. Patients were followed from index date until end of continuous enrollment, death, or end of study period (12/31/15) for the assessment of HCU and costs. DLBCL-related and non-DLBCL-related HCU and costs incurred during follow-up were evaluated. DLBCL-related HCU and costs were medical claims with a primary diagnosis of DLBCL or DLBCL-related treatment (chemotherapy, radiation, stem cell transplant [SCT], supportive care) and pharmacy claims for DLBCL treatment. Proportions of patients with HCU were reported. Costs were calculated as per-patient-per-month (PPPM) costs and reported as mean and standard deviation (SD). Patients with a capitated payment plan were excluded from the cost analysis.

**Results:** 1,267 treated DLBCL patients were identified. Over the follow-up period, 66.0% of patients had ≥1 inpatient admission, with more patients having a non-DLBCL-related than DLBCL-related admission (Table 1). 60.0% of patients had ≥1 emergency room visit over the follow-up period; visits were predominately for non-DLBCL-related. Nearly all patients had ≥1 physician office visit (92.4%) and other outpatient visits (99.6%). The mean PPPM costs incurred during the follow-up period was $11,890 (SD: $11,515) (Table 1), and costs were higher in Year 1 ($14,402, SD: $10,951) than in Year 2 ($4,190, SD: $8,076). About 55% of costs overall were related to DLBCL medical services ($6,532 PPPM, SD: $6,457). DLBCL-related medical PPPM costs decreased substantially from Year 1 ($8,327, SD: $5,925) to Year 2 ($1,443, SD: $4,349). This decrease was driven by the decreases in chemotherapy and supportive care medical services from Year 1 to Year 2. Non-DLBCL-related medical costs accounted for about 42% of the overall PPPM costs ($4,955, SD: $7,210); and a decrease was observed from Year 1 ($5,640, SD: $7,468) to Year 2 ($2,447, SD: $5,456). Inpatient admission was the main component of non-DLBCL-related costs, and associated costs decreased from Year 1 to 2.

**Table 1.**
Summary/Conclusions: The economic burden associated with the treated DLBCL population is high, with the majority of costs incurred during the first year of diagnosis. Between the first and second year of diagnosis, costs decrease mainly because of the decrease in the DLBCL-related treatment costs. In addition, HCU for DLBCL-related services decreased in Year 1 vs Year 2.

PB1706
PHARMACOKINETICS OF RITUXIMAB IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA
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Background: Rituximab dosing is based on evidence from clinical practice rather than from consideration of pharmacokinetics and factors influencing individual exposure. Clinical use of rituximab can be improved through a more individualized treatment.

Aims: The objective of this investigation was to typify rituximab pharmacokinetics in 29 newly diagnosed patients with the diffuse large B-cell lymphoma who received rituximab in combination with cyclophosphamide, doxorubicin, vincristine and methotrexate/prednisolone every three weeks. The association of rituximab pharmacokinetics with clinical outcome was also investigated.

Methods: Rituximab serum levels were defined by enzyme-linked immunosorbent assay and assessed by a population pharmacokinetic analysis applying the non-linear mixed effects modelling.

Results: A 2-compartment model comprising linear non-specific clearance of 0.20% with Cmax 0.0478–0.418 at day 1 (95% CI: 0.181 – 0.390) was observed. The target-specific drug disposition of rituximab was recognised to describe the data. The non-specific clearance was found to be lower in older patients and those with lower body weight. Additionally, the central compartment volume was higher in males. An unambiguous association of clinical response with rituximab pharmacokinetics has been detected. The rate constant of specific clearance decay was 0.143 day⁻¹ (95% CI: 0.0478 – 0.418) in patients with no disease progression, while in patients with disease progression it was 0.82% lower (95% CI: 33.4 – 95.0).

Summary/Conclusions: These results imply that time-changes in clearance could serve as a predictive marker of response to rituximab. Our findings prove the rationale for studies evaluating higher doses of rituximab in selected patients.

PB1707
HOW 18FDG PET/CT CAN IDENTIFY BONE MARROW INFLTRATION IN PATIENTS WITH NON-HODGKIN’S LYMPHOMA
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Aims: To develop an 18FDG PET/CT-based discrimination model to detect BMI on the posterior iliac crest in 3 different PET/CT evaluation methods: 1) visual analysis, 2) quantitative analysis with bone marrow biopsy in NHL patients.

Results: A total of 393 patients (188 females/205 males) with the median age of 68 years (range 18-84) and in- and out-patients. Deficit clearance of 0.278 (95% CI: 0.181 – 0.390) L/day, corresponding to target-mediated drug disposition of rituximab was recognised to describe the data. The non-specific clearance was found to be lower in older patients and those with lower body weight. Additionally, the central compartment volume was higher in males. An unambiguous association of clinical response with rituximab pharmacokinetics has been detected. The rate constant of specific clearance decay was 0.143 day⁻¹ (95% CI: 0.0478 – 0.418) in patients with no disease progression, while in patients with disease progression it was 0.82% lower (95% CI: 33.4 – 95.0). Bone marrow involvement was present in 68 patients (17.3%). Low IPI risk was present in 194 patients (49.4%), low intermediate in 86 (21.9%), high intermediate in 77 (19.6%), and high in 36 (9.2%). Median absolute lymphocyte count (ALC) at diagnosis was 1.35x10⁹/l (95% CI: 0.07-0.67x10⁹/l), absolute monocyt e count (AMC) was 0.64x10³/l (95% CI: 0.06-8.5x10³/l), AL/CAMC was 2.3 (range 0.07-3.7x10⁹/l), hemoglobin level was 125g/l (range 57-421g/l), platelet level was 274x10⁹/l (range 50-584x10⁹/l), C-reactive protein was 10.2 mg/l (range 0.10-438mg/l), erythrocyte sedimentation rate (ESR) was 30mm/h (range 2-636mm/h), and albumin level was 38g/l (range 20-51g/l). Complete remission (CR) was achieved in 288 patients (73.3%), partial remission (PR) in 58 (14.8%), stable disease (SD) in 5 (1.3%) and progressive disease in 42 (10.7%). Disease relapse was confirmed in 59/346 patients (17.0%). OS was influenced by the presence of B symptoms (p<0.004, 95% CI: 1.26-3.67), ECOG ≥2 (p<0.001, 95% CI: 1.827-4.290), Ann Arbor clinical stage (p<0.0001, 95% CI: 1.601-3.883), and albumin level (p<0.0001, 95% CI: 0.905-0.953). Optimal cut point for albumin level was 34g/l, and was determined by Receiver operating characteristic (ROC) curve (AUC 0.699, 95% CI: 0.629-0.770; p<0.0001). The prognostic value of IPI was highly statistically significant for OS (p<0.0001, 95% CI: 1.545-2.236). However, other analyzed parameters did not influence OS. Multivariate analysis among significant parameters (presence of B symptoms, IPI, and albumin), has pointed to IPI (HR 1.81, p<0.0001, 95% CI: 1.489-2.222), and albumin level (HR 1.77, 95% CI: 1.164-2.69, p=0.008) as the most important parameters that influenced survival.

Summary/Conclusions: Although IPI is widely used as a prognostic index in DLBCL, it cannot fully recognize high-risk patients. Pretreatment albumin level may represent a useful tool in order to discriminate high-risk patients and is likely to add significant information to the IPI.

PB1709
TREATMENT PATTERNS AND TREATMENT RESPONSE IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA IN ROUTINE CLINICAL CARE IN THE UNITED STATES – A CLAIMS DATABASE STUDY
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Background: DLBCL is the most common histologic subtype of non-Hodgkin lymphoma. Treatment guidelines recommend rituximab in combination with chemotherapy as first-line therapy (1LT). For patients who are refractory or relapse, higher dose chemotherapy with stem cell transplant, combination chemotherapy, or single-agent rituximab are recommended in subsequent lines.

Aims: To compare real-world treatment patterns of patients with newly diagnosed DLBCL to NCCN guideline recommendations.
TP53 GENE MUTATIONS IS A PREDICTOR OF HIGH GRADE B-CELL LYMPHOMA PROGRESSION

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Background: High grade B-cell lymphoma (HGBL) is subdivided on poor prognosis double-hit (DH) and not otherwise specified (NOS) variant, which appears sometimes with primary refractory behavior. Mutations in TP53 gene (MUT-TP53) lead to blockage of apoptosis in cells and appearance of additional oncogenic events contributing to tumor progression. Correlation between presence of MUT-TP53 and anti-tumor response in patients with HGBL is unclear.

Aims: To evaluate an effect of MUT-TP53 on survival parameters of patients with high grade B-cell lymphoma.

Methods: Since 2005 to 2017 years in FGBU National Research Center for Hematology Russian Federation detection of high grade B-cell lymphoma were established in 47 patients: 13 – double hit, 34 – not otherwise specified. We had available biologic samples from 32 pts with HGBL: 11 double-hit and 21 – NOS HGBL. 19 pts underwent courses of intensive treatment. Some patients were untreated; therefore, subsequent studies should explore reasons for lack of treatment.

Results: Of the 2,216 patients selected into the study, 1,267 (57.2%) initiated 1LT, and median (interquartile range [IQR]) time to therapy was 0.7 (0.4–1.1) months. The majority of patients received combination (87.7%) vs single-agent (12.3%) chemotherapy. R-CHOP (60.5%) was the most frequently used combination chemotherapy, while rituximab monotherapy comprised 67% (8.2%) of single-agent use in 1LT. Median (IQR) duration of 1LT was 4.2 (2.3–4.5) months. At the end of 1LT, 64.0% (n=811) had evidence of remission, 15.0% (n=190) progressed, and 1.2% (n=15) had no evidence of remission. Second-line therapy (2LT) was initiated by 158 patients who progressed after 1LT; 29.6% received a single agent, and 70.4% received combination chemotherapy. In 2LT, rituximab (12.6%) remained the top single agent used, while bendamustine+rituximab (15.7%) and R-CHOP (8.2%) were the most common combinations; 82% of patients received stem cell transplant. Median (IQR) duration of 2LT was 2.1 (1.2–3.8) months. Of the 2LT patients, 44.0% (n=70) had evidence of remission, 26.4% (n=42) progressed, and 3.1% (n=5) had no evidence of remission. 34 patients who progressed after 2LT received third-line therapy (3LT); 29.4% received a single agent, while 70.6% received combination chemotherapy. In 3LT, rituximab (5.9%), etoposide (5.9%), and carboplatin (5.9%) were the most common single agents, while bendamustine+rituximab (20.8%) and etoposide+carboplatin+rituximab (17.6%) were the most common combinations; 8.8% of patients received stem cell transplant. Median (IQR) duration of 3LT was 3.5 (0.9–5.2) months. Following 3LT, 32.4% (n=11) had evidence of remission, 29.4% (n=10) progressed, and 5.9% (n=2) had no evidence of remission.

Summary/Conclusions: DLBCL treatment in routine clinical care aligns with guidelines, most patients receiving rituximab in combination with chemotherapy. A small proportion of patients received single-agent chemotherapy in 1LT. As expected, remission rates decreased with subsequent lines of therapy. Some patients were untreated; therefore, subsequent studies should explore reasons for lack of treatment.

PB1710

TP53 GENE MUTATIONS IS A PREDICTOR OF HIGH GRADE B-CELL LYMPHOMA PROGRESSION

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Results: Mutations in TP53 GENE MUTATIONS IS A PREDICTOR OF HIGH GRADE B-CELL LYMPHOMA PROGRESSION were detected in 9 pts (n=9). 6 pts (c.517G>A) were 22.4% p.V173M (p.175C>G 17.6%, p.R175P c.517G>A 22.4% p.V173M). Pts and their relatives hadn’t a history of primary multiple tumors. Seven from nine pts with MUT-TP53 had MYC-R (3-double hit, 4-single hit lymphoma). Groups of pts with WT-TP53 and MUT-TP53 were comparable for main clinical characteristics. According to results of univariate analysis, patients with MUT-TP53 had lower duration of overall survival a higher probability of disease progression. Thus, median of overall survival in pts with c MUT-TP53 was 7.0 (3.5 - 40.9) vs 30.5 (0.6 - 160.9) months in patients with WT-TP53, (p=0.03). Median time to disease progression in pts with c MUT-TP53 was 3.5 (0.3 - 16.1) vs 30.5 (0.6 - 160.9) months in patients with WT-TP53, (p=0.00016). In multivariate analysis, MUT-TP53 was an independent factor of early disease progression in HGBL independently of double-hit status (Figure 1).

Figure 1.

Summary/Conclusions: Mutations in TP53 gene - a significant predictive factor of early disease progression in high grade B-cell lymphoma.

PB1711

HTLV-1 INFECTION INCREASED THE RISK OF OTHER MALIGNANCY

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Background: The correlation between HTLV-1 infection and malignant neoplasm other than ATL remains unknown. Some previous studies have indicated that the frequency of primary malignant neoplasms in patients with HTLV-1 seropositive is higher than HTLV-1 seronegative.

Aims: To clarify the correlations between HTLV-1 infection and malignant neoplasms other than ATL.

Methods: We retrospectively analyzed 203 patients with HTLV-1 seropositive who were diagnosed between 2006 and 2015 at Kansai Medical University Hospital.

Results: Among 203 patients (median age 62 years: range 19 to 86 years), 43% was carrier and 57% was diagnosed with ATL. According to clinical subtype, 5% was chronic, 38% was smoldering, 28% was acute, 29% was lymphoma type. Median overall survival was 30 months in carrier, 10 months in acute, 8 months in lymphoma, and smoldering was not available. In all HTLV-1 seropositive patients, the occurrence of primary malignant neoplasm was 32%, they were all carrier or smoldering. Among them, 53% was hematology malignancy (T cell lymphoma; 41%, B cell lymphoma; 29%, MPN; 18%, MDS; 12%). Solid tumor was 47% (lung cancer; 33%, prostate cancer 13%, colon cancer; 13%, renal cell cancer; 13%). Four patients with HTLV-1 carrier who developed primary malignant neoplasm received standard chemotherapy for the neoplasm, and after the chemotherapy they developed 3 acute type and 1 smoldering type ATL.

Summary/Conclusions: In our cohort, the occurrence of primary malignant neoplasm with HTLV-1 seropositive patients was significantly high. Chronic HTLV-1 infection might associate with reduction of cytotoxic T cells and an increased risk of developing other malignancy. Furthermore, cytotoxic chemotherapy for primary malignant neoplasm might reduce cytotoxic T cells for HTLV-1 and exacerbate ATL conditions.

PB1712

Abstract withdrawn.

PB1713

THIOTENA BUSULFAN CYCLOPHOSPHAMIDE, A TOXIC CONDITIONING FOR AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CENTRAL NERVOUS SYSTEM LYMPHOMA: REMISSION OR INFECTION

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Background: CNSL represent 4% of central nervous system (PCNSL) and secondary CNS lymphoma (SCNSL) occur in 7% of systemic lymphoma. Overall survival (OS) and progression free survival (PFS) have dramatically increased in PSNL since the introduction of Methotrexate high doses and ASCT usually conditioning with TBC (Thiotepa, Busulfan and Cyclophosphamide). The studies usually tend to recommend TBC/ASCT in front line for patients under 65 years with CNSL with very few prospective data about this strategy.

Aims: We report in this multicenter retrospective study our experience concerning TBC/ASCT and its main toxicities.

Methods: All patients treated with TBC/ASCT for PCNSL or SCNSL from August 2010 to November 2016 in our centers were researched by using CHIMIO® software. TBC combined Thiotepa (250mg/m²/d from d-9 to d-7), Busulfan (3.2mg/kg/d from d-6 to d-5 and 1.6mg/kg/d on d-4) and Cyclophosphamide (60mg/kg/d on d-3 and d-2) followed by ASCT transplantation at d0. Clinical data were extracted from the medical records. We measured OS and PFS from the date of ASC/T and transplant related mortality (TRM) (defined by death occurred 3 months after ASC/T).

Results: 24 patients, without any major co-morbidity, were included. Median age at ASC/T was 58 years (23-66). 22 of 24 were DLBCL and 2 follicular lymphoma and there were 15 PCNSL and 9 SCNSL. All but one, received 1 or 2 lines of chemotherapy (with high doses Methotrexate in first or second line) before ASC/T. 15 were in complete response (CR) and 9 in partial response (PR) before TBC/ASCT. Median duration of hospitalisation was 33 days (15-78) and of aplasia was 14 days (7-37). Median follow-up was 10 months (0-73). At the end of follow up 5 patients have died. Among the 3 patients older than 60 years in PR before ASC/T, no one survived. At 1 year, OS was 52.5% in the 2nd group (40 patients, 38.5%) and 12 patients (12.5%) treated with R-DA-EPOCH were included in the 2nd group (40 patients, 38.5%) and 12 patients (12.5%) treated with R-DA-EPOCH were included in the 3rd group. Significant difference between the groups was observed by the age (younger patients in the 3rd group, p=0.042) and stages distribution (early stages were more common in the 1st group, p=0.05). CRs were 61.5% in the 1st group, 52.5% in the 2nd group and 83.3% in the 3rd group (p=0.01). CR was 17.3%, 42.5% and 83.3%, respectively (p<0.001). 2-year PFS was 44±1±3% in the 1st group, 74,1±8,8% in the 2nd group and 88±10,5% in the 3rd group (p=0.09). 2-year OS was 51.8±8,7%, 54,8±10,2% and 87.5±11,7%, respectively (p=0.197).

The rate of neutropenia, thrombocytopenia and hepatotoxicity were comparable in three groups. Neutropenia, febrile neutropenia and cardiotoxicity were less common in the group treated with R-DA-EPOCH (p=0.05, p=0.01, respectively). Neurotoxicity was more frequent in this group (p=0.043).

Summary/Conclusions: The level of ORR and CRR was significantly higher in the R-DA-EPOCH group, as well as the 2-year OS between the groups. Toxicity was acceptable in all groups. Levels of neutropenia, febrile neutropenia and cardiotoxicity were less common and neurotoxicity was more frequent in the R-DA-EPOCH group. Thus, R-DA-EPOCH could be considered as the most efficient treatment regimen in patients with DLBCL from high and high-intermediate risk groups.
are no established predictors of prognosis. Although serum soluble interleukin-2 receptor (sIL-2R) levels are associated with clinical outcomes in newly diagnosed patients with PTCL-NOS, it remains unclear whether sIL-2R levels can predict prognosis in patients with relapsed/refractory PTCL-NOS.

Aims: This study evaluated whether sIL-2R levels at the time of salvage chemotherapy were associated with prognosis in cases of relapsed/refractory PTCL-NOS.

Methods: We retrospectively analyzed 45 patients with relapsed/refractory PTCL-NOS who received salvage chemotherapy at our institutions (1996–2016). All patients received CHOP or CHOP-like therapy as their initial treatment. The primary outcome was defined as overall survival (OS), which was calculated from the date of the salvage chemotherapy to the date of death from any cause or the last follow-up.

Results: The median age at salvage chemotherapy was 68 years (range: 37–86 years). The median serum sIL-2R level was 3,476 U/mL (range: 280–24,400 U/mL). Receiver operating characteristic curve analysis revealed that the optimal sIL-2R cut-off value for predicting OS was 2,283 U/mL (area under the curve: 0.672, 95% confidence interval [CI]: 0.421–0.923). Thus, we defined patients with serum sIL-2R levels of ≥2,283 U/mL as the high sIL-2R group and the other patients as the low sIL-2R group. The two groups had similar clinical characteristics at the salvage chemotherapy, with the exception of their international prognostic index (secondary IPI) and performance status (PS). The high sIL-2R group had significantly higher secondary IPI (≥2 vs ≤1) and poorer PS (≥2 vs ≤1) at the time of the analysis. The median follow-up was 4.9 years (range: 16 months to 5 years). The 2-year OS in the low sIL-2R group was 55.6±17 years, and the 2-year OS in the high sIL-2R group was 36.3±64 years (p<0.05). The PFS for patients with and without autoimmune diseases were 44.3±32.1 months and 50.9±28.6 months, respectively (mean±standard deviation; p=0.334). These two groups of patients had similar OS time as well (46.4±31.5 months vs 52.9±28.0; mean±standard deviation; p=0.337). Univariate analysis did not show autoimmune diseases were associated with inferior OS in lymphoma patients (crude hazard ratio: 1.32; 95% confidence interval: 0.89–2.0; p=0.178). The PFS for patients with and without autoimmune diseases were 44.3±32.1 months and 50.9±28.6 months, respectively (mean±standard deviation; p=0.334). These two groups of patients had similar OS time as well (46.4±31.5 months vs 52.9±28.0; mean±standard deviation; p=0.337). Univariate analysis did not show autoimmune diseases were associated with inferior OS in lymphoma patients (crude hazard ratio: 1.32; 95% confidence interval: 0.89–2.0; p=0.178).

Summary/Conclusions: Our study showed that sIL-2R at the time of salvage chemotherapy was a potential predictor of OS and PFS in patients with PTCL-NOS. However, further studies are needed to confirm these findings in larger cohorts of patients.
Background: Primary central nervous system lymphoma (PCNSL) is a rare type of non-Hodgkin’s lymphoma. Two independent prognostic scoring systems have been developed at the Memorial Sloan-Kettering Cancer Center (MSKCC) and the International Extranodal Lymphoma Study Group (IELSG). The former considers age and Karnofski’s performance status (PS) as prognostic parameters (JOC: 2006;24:571). The latter includes age, Eastern Cooperative Oncology Group (ECOG) PS, the presence of deep lesions, serum lactate dehydrogenase (LDH) and total protein levels in the cerebrospinal fluid (CSF) (JCO 2003;21:286). Neither of the two systems has been verified in the Asian population, leading to concerns regarding applicability in this region.

Aims: This study was conducted to test the prognostic power of the 2 systems in PCNSL patients in Taiwan. In addition, we analyzed the parameters of the IELSG system to figure out the most powerful prognostic factors and then established a new scoring system.

Methods: The medical records of patients with tissue-proven PCNSL were retrieved from 15 academic hospitals in Taiwan through January 2002 to December 2011. They were stratified into different groups according to the MSKCC or the IELSG system and the overall survivals (OS) were evaluated. All parameters in the IELSG system were checked by multi-variable analysis to establish a new scoring system.

Results: When the IELSG scoring system was applied, the 2-year OS in low, intermediate and high-risk groups were 78.3%, 43.9% and 37.5% respectively with a crossover in the latter 2 groups (Figure 1A). When the patients were stratified by the MSKCC scoring system, the 2-year OS of class I, II and III were 65%, 68% and 20% (Figure 1B), respectively. We conducted single-variable analysis of the 5 parameters included in the IELSG scoring system and only age and ECOG PS were statistically significant. In the multi-variable analysis, these 2 factors were almost equally weighted. Based on these findings, we re-stratified the patients into 3 groups. Group 1 comprised patients with both age <60 and ECOG PS <2 and Group 3 with both age ≥60 and ECOG PS ≥2. The patients not fulfilling criteria of either Group 1 or Group 3 were categorized as Group 2. According to this new scoring system, the median OS of Groups 1, 2 and 3 were 1,573, 548 and 304 days (Figure 1C), respectively, and their OS curves could be nicely distinguished.

Background: The incidence of lymphomas is increasing with age. Many aggressive lymphomas are now considered to be curable. All fit patients, even elders, are candidates for optimal treatment with a curative intent. Diffuse Large B Cell Lymphoma (DLBCL) is the most common non-Hodgkin Lymphoma, with 60% of curative rates after standard R-CHOP regimen. Patients that relapse can be rescued with salvage treatment in 20-30%. The elders are not considered for full standard treatment in many centers. Geriatric scales are starting to be used to stratify patients and offer them individualized treatments. The use of GSCF for neutropenia prophylaxis is not a standard of care in this population.

Aims: The objectives of this study were: 1) Validate CIRS score in a DLBCL cohort; 2) Analyse the impact of CIRS score in OS; 3) Analyse the impact of GSCF prophylaxis in neutropenic fevers.

Methods: Between November 2008 and November 2015, 41 DLBCL patients with ≥60 years at diagnosis from a single institution and homogeneously treated with R-CHOP were analyzed. Patients were evaluated for comorbidities with Cumulative Illness Rating Scale (CIRS). CIRS score was used to detect the most unfit population and evaluate the average of admissions stay and the impact on OS. The CIRS scale was adjusted by removing the hematological impact since all our patients were diagnosed with a hematologic malignancy. The cut-off point for CIRS score was selected using a ROC analysis. Neutropenic fever (NF) events were recorded and the use of GSCF in prophylaxis were analyzed, as well as the admission days for adverse events.

Results: In our series, 20 patients (48%) were males. Median age at diagnosis was 73 years old (range 60-90) With a median follow-up of 32 mo. (range 0-96), the median PFS was 51 months and the OS was 61 mo. The patients were stratified by the R-IPI and the NCCN-IPI. The ROC analysis showed a scoring of 5.5 in CIRS to identify two different risk groups, with an AUC of 70.5%, a sensitivity of 87% and a specificity of 48% (p=0.02). In the low risk group, with CIRS <6 (n=17), 7 (41%) patients were admitted with a mean of stay of 6.2 days (range 1-62) vs the high-risk group with CIRS >6 (n=24). Of this group, 11(45%) patients were admitted with a mean of stay of 10.6 days (range 1-62), p=0.035. The CIRS scale was also used to discriminate two OS groups; the low risk showed a median OS not reached vs 29 mo. the high-risk group, with a Hazard ratio of 2.68 (CI95%; 1.031-5.882, p= 0.042). NF was the most common ER visit, n=18 (36%). Of the 16 patients with NF, 10 (55%) were prescribed with GCSF prophylaxis mid cycles. Of all patients with GCSF (n=43) only 10 (24%) NF were reported. 11/17 patients (65%) who didn’t use GCSF prophylaxis had an NF episode. The Odds ratio (OR) for the patients under prophylaxis was 0.232 (CI 95%; 0.085-0.634, p=0.004) (Figure 1).

Figure 1.

Summary/Conclusions: The OS and the PFS in our sample is similar as described in larger studies. The days of admissions adjusted to the standard of care. The CIRS scale also help separate two distinct OS curves, giving physicians a new tool to help discriminate worse prognostic patients, making them good candidates for adapted therapies. The use of GSCF prophylactic can protect the elderly patients from NF, and should be used in all patients in this category.

PB1721

PRIMARY ADRENAL LYMPHOMA: A SINGLE-CENTER EXPERIENCE
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Background: Primary adrenal lymphoma (PAL) is rare, with slightly more than 250 cases currently described in the English-language literature. In current classifications, there is not yet a consensual definition of PAL. The aim of this study was to report a large single center clinical case series of primary adrenal lymphoma (PAL) in terms of clinical presentation, pathological and imaging features, and treatment outcome.

Methods: We performed a retrospective analysis of 21 patients diagnosed with PAL who presented to our center between January 2005 and January 2014.

Results: Median age at presentation was 48 years (range: 27–73) with a male-to-female ratio of 5:2. Bilateral and right-sided adrenal involvement were seen in 12/21 and 7/21 patients, respectively. Adrenal insufficiency (AI) was seen in...
6/10 evaluated patients. Computed tomography scans showed slight to moderate contrast enhancement of adrenal masses in 4/5 patients (80%), and magnetic resonance imaging identified a normal T1 and longer T2 phase. Diffuse large B cell lymphoma (DLBCL) was the most common immunophenotype (82.6%). Two patients died due to rapid disease progression before treatment. Three patients were treated with chemotherapy/external beam radiotherapy. Two patients received autologous stem cell transplantation as consolidation therapy. Two-year overall survival and progression-free survival were 54.2% and 51.0%, respectively.

Summary/Conclusions: These findings suggest that PAL should always be considered in differential diagnosis of adenral mass with AI. Moreover, DLBCL was observed as the most common histological subtype of PAL. Despite the contrasting previous reports, long-term prognosis of PAL is not necessarily inferior to that of non-Hodgkin lymphoma in general.

PB1723
HEMATOLOGICAL MALIGNANCIES IN SOLID ORGAN TRANSPLANT RECIPIENTS: RETROSPECTIVE SINGLE-CENTER ANALYSIS IN JAPAN
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Background: Mantle cell lymphoma (MCL) is a rare subtype of non-Hodgkin lymphoma that has an aggressive clinical course and poor prognosis. Although current front-line combination chemo-immunotherapies followed by autologous stem-cell transplantation (ASCT) have improved the outcomes of affected patients (pts), there is no cure. Ibrutinib is an oral covalent inhibitor of Bruton tyrosine kinase that showed significant activity in relapsed/refractory MCL in clinical trials, but in real-life routine, the efficacy and safety may not always mirror those seen in clinical trials.

Aims: We investigated the clinical use of ibrutinib as a single-agent in 31 pts with relapsed or refractory MCL to obtain additional information about predictive factors, outcomes and toxicity in a real-life context.

Methods: We studied a group of 31 pts treated (or still in treatment) with ibrutinib to assess effectiveness in terms of overall response rate, complete response rate, progression free survival and adverse events (AEs) in a real-life context. Data were collected also with reference to clinical and biological characteristics of the disease (MIPI, MIPIb, bone marrow involvement, stage, histology, presence of bulky mass and/or extranodal disease) both at the time of diagnosis and at the time of the start of ibrutinib therapy, and to the type and number of previous therapies.

Results: The median age of the ibrutinib therapy, the median age was 70 years (range, 45-62), 100% of pts had high risk MCL according to the MIPI score, 83.9% of pts had disease stage III or higher, 41.9% of pts had bone marrow involvement, and 45.2% of pts presented extranodal involvement of MCL. 26 pts were treated for relapsed MCL for refractory disease. They had received a median of 2 (range, 1-5) different treatments before ibrutinib. The overall response rate was 61.3% (9/15) for pts with ECOG 0-1 and 19.2% (2/11) for pts with ECOG 2. Despite the differences in the treatment history, the median OS was 21.8 months (range, 0.2-95.1) after the start of therapy. After 15 months, we observed 4 relapses, characterized by leukemic transformation (LT) and 6 complete responses, 1 after only 2 months of therapy, the others within 6 months of therapy. After 15 months, we observed 4 relapses, characterized by leukemic transformation (LT) and 6 complete responses, 1 after only 2 months of therapy, the others within 6 months of therapy. 6 complete responses, 1 after only 2 months of therapy, the others within 6 months of therapy. 6 complete responses, 1 after only 2 months of therapy, the others within 6 months of therapy.

Summary/Conclusions: Single-agent oral ibrutinib shows a high response rate and produces rapid responses regardless of the number and quality of previous therapies. However, the quality of life and response does not always predict a better PFS or longer duration of response. Furthermore, resistance to ibrutinib in pts with MCL is associated with fulminant, severe progression. ibrutinib is well tolerated also in real-life experience. The weight increase in 13% of pts suggests that ibrutinib may have an anabolic effect, including alteration in body composition and lipid profile. Larger cohorts of pts and longer follow-up are warranted to confirm these preliminary data.

PB1724
MYC REARRANGEMENT HAS A STRONG PROGNOSTIC IMPACT IN THE FEMALE PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA
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Background: Solid organ transplant recipients have elevated onset risks of hematologic malignancies (HMs) due to long-term administration of immunosuppressive drugs. To date, however, few studies about the incidence and impact on survival of HMs following solid organ transplantation have been conducted in Asian countries.

Aims: The aim of this study was to identify the incidence, characteristics, risk factors and prognosis of HMs in solid organ transplant recipients at our institution.

Methods: Clinical data of patients undergoing kidney, liver and heart transplantation at Hokkaido University Hospital between 2004–2016 were retrospectively analyzed. Kaplan-Meier analysis was performed for the cumulative incidence rates (CI) of HMs, graft survival and patient survival. Patient’s characteristics were compared between groups by the student t-test or K-S square test.

Results: A total of 16 cases of HMs were identified, 9 post-transplant lymphoproliferative disorder (PTLD). Of the 2016 cases, 9% were PTLD, 1 myeloproliferative neoplasm (MPN) and 1 recurrent non-Hodgkin lymphoma. The CI of PTLD were 1.1%, 1.5% at 10 years in kidney transplant recipients (n=352), 0.92%, 2.6% at 5, 10 years in liver transplant recipients (n=287) and 29% at 1 year heart transplant recipients (n=5), respectively (P<0.001). AML/MDS and MPN were not observed in recipients, and CI were 2.3% at 5 and 10 years (P<0.01). There was no difference in background factors other than transplanted organ type between recipients with HMs and without HMs. Patients with EBV-positive PTLD (n=5) were younger (P<0.05) and had less extranodal diseases (P<0.05) compared with EBV-negative PTLD (n=4). All patients with monomorphic PTLD (n=4) were treated with chemotherapy combined with rituximab and had been in remission. In patients with other PTLD, reduction or withdrawal of immunosuppressant or rituximab alone resulted in stable disease or remission. All AML/MDS but 2 acute promyelocytic leukemia in pediatric patients were chemo-refractory and lethal. 10-year OS were 92% and 100% in kidney and heart transplant recipients. In liver transplant recipients, 10-year OS were 74%, 100% and 50% in patients without disease, with PTLD and with myeloid neoplasm, respectively. Survival in adult liver transplant recipients with myeloid neoplasms was inferior to that without disease (P=0.05), 10-year graft survival rates were 72% and 57% in a lymphoma-free and lymphoma transplant recipients, respectively. Our clinical approach for myeloid neoplasms following solid organ transplantation are needed.

Summary/Conclusions: The incidence of PTLD in solid organ transplant recipients in Japan is comparable to that in Western countries, whereas the incidence of myeloid neoplasms is higher in liver transplant recipients. PTLD does not have a negative impact on the prognosis of solid organ transplant recipients under appropriate management, while EBV-associated PTLD does. Our clinical approach for myeloid neoplasms following solid organ transplantation are needed.
variety analysis was performed for the OS. Elevated LDH level, stage ≥3, PS ≥2, extranodal sites, IPI=3, BCL6 negative (IHC), and MYC rearrangement (FISH) were significant factors in the female patients; however, PS ≥2 and IPI=3 were significant factors in the male patients. Univariate analysis was also performed for PFS. Elevated LDH level, PS ≥2, IPI=3, BCL6 negative (IHC), and MYC rearrangement (FISH) were significant factors in the female patients; however, PS ≥2 was the only significant factor in the male patients. Multivariate analyses were then performed using these factors in the Cox proportional hazard model. MYC rearrangement (FISH) [hazard ratio (HR): 9.13, 95% confidence interval (CI): 2.33–35.77, P=0.0015], and IPI ≥3 were identified as independent significant prognostic factor for OS in the female patients with DLBCL. Furthermore, MYC rearrangement (FISH) (HR: 2.47, 95% CI: 1.87–327.8, P=0.01494), and elevated LDH level were identified as independent significant prognostic factor for PFS in the female patients with DLBCL. On the other hand, PS ≥2 was identified as the only significant prognostic factor for OS (HR: 44.27, 95% CI: 6.71–292.2, P<0.001), but not for PFS in the male patients with DLBCL. Five of seven female patients with DLBCL and MYC rearrangement died from lymphoma progression. The median OS in the female patients with DLBCL and MYC rearrangement was 8.0 months (range: 1–35 months) compared to 21.5 months in those without MYC rearrangement (range: 1–79 months, P=0.003). On the other hand, in the male patients (n=13) with DLBCL, MYC rearrangement was not significantly associated with poor OS (Figure 1).

**Summary/Conclusions:** These results suggest that MYC rearrangement by FISH is significantly associated with very poor OS and PFS in the female patients with DLBCL but not the male patients with DLBCL. On the other hand, PS ≥2 is significantly associated with poor OS in the male patients with DLBCL.

**PB1725**

**ASSESSING THE RISK FOR PERFORATION IN DIFFUSE LARGE B-CELL LYMPHOMA INVOLVING THE INTESTINES USING COMPUTED TOMOGRAPHY CHARACTERISTICS.**

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**Background:** Around 40% of all Diffuse Large B-Cell Lymphoma (DLBCL) cases involve extra-nodal sites, the most common being the gastro-intestinal (GI) tract. DLBCL patients with intestinal involvement are particularly prone to develop GI perforation, which might be life threatening and entail significant morbidity. Identification of patients at risk for perforation may promote the performance of pre-emptive surgical resection of the involved segment. Although computed tomography (CT) scan is widely used at diagnosis, incorporation of CT results into the risk stratification of perforation has not yet been performed.

**Aims:** To determine risk factors for perforation in patients with DLBCL and intestinal involvement, with an emphasis on CT findings.

**Methods:** A retrospective single center study, including all consecutive DLBCL patients that presented with intestinal involvement between 2005 and 2016. The analysis included clinical, laboratory pathological and radiological parameters. Cases with DLBCL of the stomach were excluded.

**Results:** Forty-nine cases (30 men, 19 women) were included. Median age of the entire cohort was 64 years (54.5–77 IQR). Early stage (1, 2) according to the Lugano system was reported in 35% of cases. Small intestine involvement was more frequent (50%), followed by large intestine and ileocecal (32% and 16%, respectively). Forty-three (88%) patients underwent CT scan at diagnosis. Most lesions were defined radiology as concentric (n=27, 63%) (as opposed to eccentric), and transmural (n=31, 74%) (as opposed to non-transmural). Of note, 98.3% of the 27 concentric lesions were also transmural, compared with 31% (11 of 35) of eccentric lesions. The median length and wall thickness of the involved site was 9.3 cm (5.8–13.5) and 15 mm (10–20), respectively. Ten (20%) patients developed an intestinal perforation. Six of the perforations (60%) involved the small intestine, 3 (33%) occurred at diagnosis prior chemotherapy, and 4 (40%) occurred within the first 21 days post therapy. All perforated lesions were eccentric and transmural, with a median length of 11.2 cm. Eight (80%) patients underwent an urgent operation due to GI perforation, including 3 that resulted in an ostomy. Perforation led directly to 2 (20%) deaths. Perforation resulted in delayed administration of chemotherapy in 50% of cases (n=5). A univariate regression analysis found a higher risk of perforation in patients presenting with a concentric lesion (p=0.001, HR=34.6, CI 25.9–53.3) and a longer involved GI segment (p=0.008, HR=1.06, CI 1.017–1.166). Each extra centimeter to the length of the GI segment involved was associated with a 6% increase in the risk for perforation. There was no association between sex, age, performance status, hemoglobin, LDH, albumin, iron, ferritin, K/β7, disease stage, anatomical location nor the involved site wall thickness and risk of perforation.

**Summary/Conclusions:** DLBCL patients presenting with an involvement of a long intestinal segment, especially with a concentric, transmural lesion, are at higher risk for perforation. These patients should be considered for a preemptive surgical resection, dependent on lesion site and operative risk.
Summary/Conclusions: 1) The incidence of double or triple hit lymphomas in our institution is consistent with the literature. 2) The most common regimen used in double or triple hit patients was anthracycline-containing chemotherapy achieving more than 50% of overall responses in our series. Nevertheless, the majority of patients relapse, showing a short PFS and worse outcome than DLBCL without double or triple hit, as reported previously.

PB1727

EFFECTIVE TREATMENTS ARE REQUIRED FOR PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA WITH PRIMARY REFRACTORY DISEASE

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Background: DLBCL is a heterogeneous disease; it has been described that around 30% of patients present a refractory/relapsing disease following R-CHOP treatment. Rituximab-containing salvage chemotherapy followed by high-dose therapy and autologous stem cell transplant (ASCT) in chemorefractory patients remains the standard of care for these patients.

Aims: We aimed to study the clinical features and outcome of patients diagnosed of DLBCL, homogeneously treated with R-CHOP/R-CHOP-like first line regimen, who have primary refractory disease (PRD).

Methods: Three hundred and sixty-seven patients were diagnosed of DLBCL between January 2004 to August 2016 in our center. 317/367 (86.3%) were treated with R-CHOP or R-CHOP-like in first line. Forty-four (13.9%) patients had PRD and 39 (12.3%) progressed during the follow up. Survival curves were estimated using the Kaplan-Meier method and compared using the Log-Rank test. Univariate analyses were performed by Chi square test and multivariate analyses by Cox proportional hazard regression model.

Results: Among the 44 primary refractory patients, 15 (34%), with a median age of 76 years (range 63-91), were considered unfit, 11 received supportive care and 4 were treated with palliative chemotherapy (cyclophosphamide and prednisone). Twenty nine (66%) were eligible for salvage therapy and 4 were treated with palliative chemotherapy (cyclophosphamide and prednisone). Twenty nine (66%) were eligible for salvage therapy and 4 were treated with palliative chemotherapy (cyclophosphamide and prednisone). Twenty nine (66%) were eligible for salvage therapy.

Summary/Conclusions: Patients with DLBCL refractory to first line R-CHOP are not rescued with current salvage therapies, and in this setting DLBCL must be considered an incurable disease with a very short survival, similar to that of patients treated with palliative care. Patients with B symptoms and elevated LDH at diagnosis have a significantly higher risk to be refractory to R-CHOP. It is imperative to identify early these patients and to design new therapies for them.

PB1728

RITUXIMAB BENDAMUSTINE CYTARABINE IS A FEASIBLE AND SAFE INDUCTION REGIMEN PRIOR TO ASCT IN FRONTAL MCL: A SINGLE CENTER RETROSPECTIVE REAL LIFE EVALUATION

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Background: Mantle cell lymphoma (MCL) is an uncommon, still incurable subtype of non Hodgkin lymphoma. The routine use of high dose Cytabrine and high dose chemotherapy followed by autologous stem cell transplant (ASCT) markedly improved the outcome and has become the standard treatment for fit, young (<65 years) patients. Recently, a large phase III trial demonstrated that Rituximab, Bendamustine and Cytabrine (RBAC) combination has a remarkable activity with a favorable safety profile both in untreated and relapsed/refractory elderly MCL patients (Visco et al., 2013 and 2017). These studies suggested that RBAC combination (with Cytabrine 800 mg/mq) is safe enough as a consolidation regimen after ASCT and feasible for fit elderly patients. Our group demonstrated that Rituximab, Bendamustine and Cytabrine (RBAC) combination has an encouraging safety profile. Further investigations are needed to assess the optimal role of RBAC within the standard first line treatments.

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Summary/Conclusions: In as the relapsed/refractory setting and in MCL patients ineligible for high dose chemotherapy, RBAC has been proven to be an efficacious induction and mobilization regimen also in transplant eligible MCL patients with an encouraging safety profile. Further investigations are needed to assess the optimal role of RBAC within the standard first line treatments.
Aims: The objective of this retrospective, observational study is to evaluate the efficacy and safety of liposomal cytarabine in patients with CNS infiltration by haematological malignancies.

Methods: 36 consecutive patients with haematological disease and risk of CNS infiltration underwent flow cytometry (FC) analysis of CSF in a single center from December 2014 to December 2016. CNS involvement was assessed by neurology, imaging (CT, MRI) and CSF analysis. Along with systemic therapy, all patients considered positive were treated 50 mg of IT Liposomal cytarabine administered by lumbar puncture every 2 weeks for 4 doses and every 4 weeks thereafter. Concomitant dexamethasone for arachnoiditis prophylaxis was added both i.v. and IT. We analysed the rate of adverse events (AE) and the time for CSF clearance. Short follow up precluded assessment of cumulative incidence of CNS relapse/progression.

Results: Data from 36 patients were analysed. A total of nine patients were considered to have CSF involvement, all of them detected by FC. Of note, all of them were considered negative for CSF infiltration by standard cytology. Three additional patients had adverse events without therapy as a consequence of MRI proven brain involvement by the malignancy. The median age of this 12 patients was 52 years (range 16-69), 58.3% were female. Diagnosis were B-cell lymphoproliferative disorder 41.7% (CLL, Burkitt, DLBCL), ALL 25%, AML 25% and multiple myeloma 8.3%. Median number of doses per patient was 6.5 (SD 1.7). CNS clearance time was achieved after a median of 1 dose (range 1-3) or 20 days (range 16-86). Overall rate of CNS response was 100%. Two patients (16.7%) had leptomeningeal relapse during the IT treatment. The overall AE incidence was 66.7%. The most common AE include: headache, peripheral sensory neuropathy, back pain and nausea. Severe neurotoxicity has been encountered in four patients: cauda equina syndrome, arachnoiditis and leptomeningeal involvement by lymphomatous tissue (1). Treatment had to be discontinued in 3 patients because of side effects but this did not lead to relapse. The median time to AE occurrence was 6 cycles (range 4-7) or 110 days (range 33-227). The incidence and severity of AE seemed to increase with the cumulative number of cycles administered. In most patients neurological complications improved or resolved with time.

Summary/Conclusions: use of liposomal formulation of cytarabine for IT administration has become an effective option for the treatment of leptomeningeal involvement by haematological malignancies. Neurological AE are reversible; however, they accumulate and worsen with time, thus precluding long-term use.

PB1732

RETROSPECTIVE EVALUATION ON EFFICACY FOR OLDERLY PATIENTS WITH STAGE 3 AND 4 DISEASE HIGH-GRADING DLBCL WITH REDUCED CYCLES OF R-CHOP OR R-GCVP: A 7 YEARS SINGLE-INSTITUTE EXPERIENCE

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Background: The most common high-grade lymphoma malignancy in adults is Diffuse Large B-Cell Lymphoma (DLBCL), which has an increasing incidence with age (1). Over 40% of patients with DLBCL are above the age of 70, and the comorbidities in this age-group present significant challenges and complexities with regards to selecting and implementing treatment regimens (2).

Aims: We present a retrospective analysis of outcomes for patients with high-grade DLBCL (stage 3 or 4 disease) who have received fewer than 6 cycles of full dose R-CHOP or R-GCVP because of poor tolerability or disease progression with treatment.

Patients and Methods: Retrospective data were collected from the cancer registry for all newly-diagnosed DLBCL patients who received R-CHOP or R-GCVP chemotherapy, with data collected from Jan 2010 to Feb 2017 from Ipswich Hospital NHS trust, United Kingdom. Patients who completed 6 cycles of chemotherapy were excluded. Interim PET-CT scan/staging CT scan was done to assess the disease response to therapy after 2 cycles of chemotherapy. The main baseline characteristics collected were age, sex, ECOG Performance Status, Ann-Arbor Stage and IPI risk stratification. The primary end point was progression standard of care (PFS) from completion of treatment. Secondary end points were overall response rate (ORR), overall survival (OS), and the reasons for premature ceasing of treatment based on graded toxicity according to NCIC-CTCAE 4.0.

Results: Of 87 patients, 12 patients were identified that fulfilled the inclusion criteria. The median age of patients was 72 years (range: 64-88 years), sex distribution was 7 male: 5 female, ECOG PS was 0-2 in 10 (83%) and ≤3 in 2 (17%) of the patients, Ann-Arbor Stage was 3 in 6 patients (50%) and 4 in 6 patients (50%), and IPI score was 3 in all 12 patients. 11 patients received R-CHOP and 1 patient received R-GCVP. The median length of treatment was 3.5 years (range: 1-6 years) and the median number of cycles administered was 75% at end of treatment assessment scan. The complete and partial response rates at the end of the treatment were 58% and 17% respectively. Progression free survival was 73% at 2 years (8 out of 11 patients) and 50% at 3 years (4 out of 8 patients). The median overall survival of deceased patients was 9.5 months (range: 2-42 months) and the median overall survival of living patients was 40.5 months (range: 27-84 months). The most common reasons for stopping the treatment were intolerance of side-effects (4 out of 12) or neutropenic sepsis (3 out of 12). 2 out of 12 patients received an incomplete course of chemotherapy due to non- compliance with treatment.

Conclusions: DLBCL treated with less than 6 cycles of full dose R-CHOP or R-GCVP chemotherapy may achieve sustained long-term remission in selected patients with high IPI and significant co-morbidity. Further research on disease characteristics including molecular profile is needed to elucidate selected populations who may achieve long-term remission with shorter cycles of chemotherapy. Further insights may derive, for example, from analysis of polymorphism of folate pathway genes and/or of NF-kb, which have been previously suggested as pharmaco-genomic targets in lymphoid neoplasms. A risk stratification model needs to be developed to reduce drug toxicity and other short and long term treatment related complications so as to improve patient experience, and pharmacoeconomic benefits.
phomas (DHL) and double or triple-protein-expression lymphomas (DPLs, TPLs) display a worse outcome. R-CHOP, which is the frontline treatment for DLBCL, showed a poor outcome in high risk IPI patients and DHLs or DPLs. From January 2011 in our centre (IRCCS AOU San Martino Hospital—IST, Genoa, Italy) R-CODOX-M/IVAC regimen has been adopted as first line in patients with aggressive DLBCL, defined by at least one among these features: high tumour burden, DPLs, IPI score ≥3 or by the presence of at least 1 extranodal site.

Aims: Our aim was to define the efficacy and feasibility of this frontline strategy and eventually identify the subgroups of patients who may benefit from this approach.

Methods: We retrospectively analyzed 20 patients affected by aggressive DLBCL treated with R-CODOX-M/IVAC. R-CODOX-M consists of rituximab 375 mg/sqm day 1, cyclophosphamide 800 mg/sqm day 1, 200 mg day 2-5, doxorubicin 40 mg/sqm day 1, vincristine 1.4 mg/sqm, methotrexate 6700 mg/sqm. IVAC-R contains rituximab 375 mg/sqm, iपosphamide 1500 mg/sqm day 1-5, etoposide 75 mg/sqm day 1-5, cytarabine 2000 mg/sqm bid day 1-3. In both cycles CNS prophylaxis was administered. According to Ann Arbor classification, 11 patients were on stage IV, 1 on stage I, 3 in stage I and 5 in stage I. Twelve patients had B symptoms. Median IPI score was 3. Eleven patients had DPLs and 4 of them had TPLs. Overall survival (OS) was calculated from the time of diagnosis to the time of death or last follow-up.

Results: After a median follow-up of 28 months, 5 patients died (25%). OS at six and twelve months was 89.4 and 70.4%, respectively, median not reached (NR). Complete remission was achieved in 11 patients (55%), partial remission in 2 patients (10%). The overall response rate was 82%. Three patients (18%) were ongoing DPLs. OS at six months was 88.9 and 64.8%, respectively, not significantly lower than non DPLs patients (p=0.77). Median OS in patients with Ann Arbor stage III or IV, OS at six and twelve months was 90.9 and 60.6%, median NR. In patients with IPI score ≥3, OS at six and twelve months was 78.6 and 45% (median 12 months). The main toxicity during CODOX and was grade ≥2 mucositis, 63% of patients. Infections occurred in 71% of patients. Renal and liver toxicity was mainly of low grade and was observed respectively in 38% and 50% of patients. Median severe neutropenia was 4.5 days (range 0-16) and median severe thrombocytopenia was only 1 day (range 0-21). Most patients (56%) needed transfusion support. In IVAC regimen, 70% of patients had the highest clinical one with 7 days of median duration of severe neutropenia (range 3-10), and 7 days (range 6-23) of thrombocytopenia. Seventy-five patients required transfusion support. Infections occurred in 42% of patients. We observed few case of grade ≥2 mucositis (17%), renal toxicity (8%) and liver toxicity (17%).

Summary/Conclusions: R-CODOX-M/IVAC is a generally well tolerated regimen, with acceptable toxicity profile in the setting of aggressive DLBCL. Results in our cohort suggest a potential benefit for DPLs, whereas higher IPI scores do not significantly impact the risk factors for survival. The next step of the study will be retrospective FISH evaluation of C-MYC, BCL2 and BCL6 translocations, for lacking patients in our cohort, in order to disclose a potential benefit for double or triple hit lymphomas.

PB1734

STOMACH DIFFUSE LARGE B-CELL LYMPHOMA: A SINGLE CENTER EXPERIENCE

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Background: Primary gastric diffuse large B cell lymphoma is a rare type of diffuse large B cell lymphoma. Immunohistochemistry followed by consolidation radiation is the standard treatment. However, the cycles of chemotherapy and the role of consolidation radiation are still under debate.

Aims: To review and analyze the treatment experience of newly diagnosed primary gastric diffuse large B cell lymphoma. We presented the treatment outcome of our institution.

Methods: We retrospectively reviewed medical records from Jan 2005 to Dec 2014 from our institution. 30 patients with primary gastric diffuse large B cell lymphoma were included. Clinical characteristics, treatment regimens, treatment response, treatment modality, and survival were analyzed.

Results: From Jan 2005 to Dec 2014, there were 30 patients with primary gastric diffuse large B cell lymphoma. Median age was 65 years of age. 53% (n=16) of patients were male. All 30 patients (100%) have received chemotherapy. 13 of them (43%) have received involved field radiation therapy (IFRT). RCHO or RCEOP was administered in 86% (n=26) of patients. Complete response (CR) rate was 80% (n=24), 5-year survival was 69%. In patients who achieved complete response (CR), the 5-year survival for 4 cycles of chemotherapy was 88% vs 86% (p=0.42), respectively. For addition of IFRT in CR patients, 5-year survival for IFRT vs no IFRT were 83% vs 90% (p=0.93), respectively. Treatment-related mortality (TRM) was 13% (n=3) and primary relapse incidence of disease was 10% (n=3). Of all of them are non-CR patients. Gastrointestinal bleeding which required admission occurred in 10% (n=3) of patients. In patients who developed GI bleeding, 2 of them were non-CR patients and they all died. No patient died of disease relapse after complete response.

Summary/Conclusions: In our series, the 5-year survival was good. In patients who achieved CR, cycles of chemotherapy and consolidation radiation did not make significant difference to the survival. Prevention of early mortality may improve the outcome of this disease. Gastrointestinal bleeding in treatment is rare but with high mortality.

PB1735

IMMUNOHISTOCHEMISTRY BIOMARKERS IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA: A RETROSPECTIVE STUDY

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Background: Diffuse Large B-cell Lymphoma (DLBCL) is a heterogeneous group with variable clinical and biological features. The International Prognostic Index (IPI) is the most important tool to identify subgroups with different survival, however, certain biological markers seem to have a prognostic value relevant and independent of IPI.
Aims: To analyze the evolution of patients diagnosed with DLBCL and the expression of BCL-2, BCL-6 and MYC.

Methods: We conducted a retrospective study that included hospitalized patients with de novo CD20+ DLBCL, with expression of BCL2+, BCL6+, BCL2/BCL6, MYC/BCL2, MYC/BCL6 treated with regimens containing rituximab, from February 2012 to November 2016. Samples were analyzed by immunohistochemistry. Statistical analysis with the SPSS V17.0 program.

Results: We included 43 patients with a median age of 65 years (22-97), 59.5% male, 45.2% had IPI 0-2, 54.8% had IPI 3-5, 26.2% stage I-II, 73.8% stage III-IV, 61.9% had extranodal disease and 23.8% bulky disease. Ki-67 was elevated in all patients who did this evaluation (n=28). In 13 patients was identified BCL2/BCL6+ in 6, and 21 patients had co-expression of BCL2/BCL6, 1 patient had MYC/BCL2 and 1 had MYC/BCL6. The R-CHOP regimen was first line treatment in 92.8% of patients. The ORR was 82.5%, with 65% of CR, 15% PR and 17.5% PD. Of those patients who received second line treatment, 8 expressed BCL2/BCL6, 4 BCL2, 2 BCL6, 1 MYC/BCL2, and 1 MYC/BCL6. Of the 12 patients who received third line treatment 3 expressed BCL2/BCL6, 6 BCL2, and 1 MYC/BCL6. The average time to next treatment (TNT) was 5.2 months (0.5-19) for second line and 4.9 for third line. Mortality rate was 45.2%. With a median follow up of 18.6 months (3-58.6), the overall survival was 24.6 months (3-62).

Summary/Conclusions: The identification of biomarkers by immunohistochemistry is a relatively inexpensive process, which, when well elaborated and interpreted, allows to find in a safe way, subgroups of patients at high risk, who benefit from more aggressive 1st line therapy and, whenever possible, from the Inclusion in clinical trials with new drugs.

PB1736

INVESTIGATION ON TREATMENT STRATEGY, PROGNOSTIC FACTORS, AND RISK FACTORS FOR EARLY DEATH IN ELDERLY TAIWANESE PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Given that the population of elderly cancer patients, including those with diffuse large B-cell lymphoma(DLBCL), is increasing, the management of cancer in the elderly has emerged as an increasingly common problem.

Aims: This study aimed to investigate the treatment strategy, prognostic factors, and risk factors of early death in elderly patients (age ≥65 years) with DLBCL in the rituximab era.

Methods: Elderly patients diagnosed with DLBCL between 2008 and 2014 were enrolled for analysis.

Results: There were 145 elderly patients with DLBCL diagnosed between 2008 and 2014. After excluding patients with primary central nervous system DLBCL (n=9) and incomplete data (n=3), a total of 133 patients (64 male and 69 female) with a median age of 74 years (range 65 to 94 years) were enrolled in the present study. Patients at a younger age and with better performance status were more likely to receive intensive frontline treatment. The median progression-free survival (PFS) and overall survival were 15 and 21 months, respectively. Anthracycline-containing chemotherapy achieved a higher remission rate and showed a trend toward better overall survival at the expense of a higher risk of severe neutropenia. Multivariate analysis revealed that very old age (≥81 years), a high-risk age-adjusted international prognostic index (aaIPI) score, and bone marrow involvement were associated with poorer PFS and overall survival. Progression of lymphoma was the major cause of death in the study population. In addition, approximately 25% of patients died within 120 days of their diagnosis. The risk factors for early mortality included very old age, a high-risk aaIPI score, and bone marrow involvement. The appearance of symptoms or signs of tumor lysis syndrome at diagnosis was associated with a trend toward early death.

Summary/Conclusions: Treatment of elderly patients with DLBCL remains a challenging and complex endeavor. Co-evaluation to tailor therapeutic interventions and offer the best supportive care may reduce complications and improve the clinical outcome of these patients.
TREATMENT OF NEWLY DIAGNOSED CENTRAL NERVOUS SYSTEM LYMPHOMA PATIENTS BASED ON COMORBIDITIES & PERFORMANCE STATUS: A SINGLE-CENTRE EXPERIENCE

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Background: Combination chemotherapy incorporating high dose methotrexate (HD-Mtx) and high dose cytarabine (Ara-C) is the standard chemotherapeutic approach for newly diagnosed primary CNS lymphoma (PCNSL). However, patients >60 years old account for 50% of cases and combining HD-Mtx with Ara-C can be associated with high toxicity and early mortality. The management of secondary CNS lymphoma (SCNSL) is less clear, but is often based upon a similar approach.

Aims: We present a tertiary centre experience in management of primary (PCNSL) and secondary CNS lymphoma (SCNSL), with therapy based on co-morbidities and performance status.

Methods: We performed a retrospective analysis of patients with a diagnosis of CNS lymphoma seen at our centre between 2011 and 2016. These were categorized into 3 groups, Group 1: treatment of newly diagnosed PCNSL prior to September 2014 where majority of patients received HD-Mtx & Ara-C combination chemotherapy, Group 2: treatment of PCNSL after September 2014 where patients were selected based on co-morbidities to receive Mtx with or without Ara-C, Group 3: treatment of newly diagnosed SCNSL. The median survival for each group was estimated using the Kaplan-Meier method and log-rank test. Overall response rates, 30 day and 90 day survival between groups 1 & 2 were compared using unpaired t test.

Results: 60 pts with a median age of 65 years old were recruited. 40 pts were diagnosed to have PCNSL at presentation, while 20 patients had SCNSL. 5 pts were excluded from this study as they did not receive any treatment. In group 1, 21 pts (84%) received combination chemotherapy incorporating HD-MTX and Ara-C. 3 pts (12%) received HD-MTX monotherapy and 1 pt (4%) received radiotherapy only. In group 2, 7 pts (53.8%) received HD-MTX and Ara-C as part of MTRix protocol or with single agent rituximab, 3 pts (23%) received HD-MTX as part of RMP protocol or with single agent rituximab, 1 pt (7.7%) received a single alkylating agent and 1 pt (7.7%) received radiotherapy only. In group 3 15 pts (88.3%) received chemotherapy incorporating HD-MTX and Ara-C, 2 pt (11.8%) received HD-MTX without Ara-C. 30 day mortality was 7 (28%) in group 1 and 0 in group 2 (0%) (p=0.03), 90 day mortality was 7 (28%) in group 1 and 2 in group 2 (15.4%) (p=0.39). Overall response rate was 9 (36%) in group 1 and 8 (61.5%) in group 2 (p= 0.13). A Kaplan Meier curve of all 3 groups is illustrated in Figure 1 below.
criteria at the outset and analyzing the possible features of laboratory TLS. Although dosing did not always follow BSH guidelines, we did respond to biochemical deterioration. The majority of patients with HD developed acute kidney injury despite rasburicase. Doses were increased in response to creatinine increases, albeit not as per guideline. It is notable that despite lower than the recommended doses of rasburicase, 6/8 patients with lab TLS did not progress to clinical TLS, and none required dialysis. The guideline is a good tool for the risk stratification and treatment of patients at risk of TLS. In clinical practice 100% compliance is hard to achieve. Responding to trends in creatinine may explain why, despite lower than recommended doses, our outcomes were still good. It would be interesting to see if further work with larger numbers of patients would support this. Since this audit was completed, the ePrescribing system has been altered to improve practice and a re-audit is planned.

PB1742

PROGNOSTIC IMPACT OF SYNCHRONOUS MULTIPLE PRIMARY MALIGNANT TUMORS ON NEWLY DIAGNOSED LYMPHOMA PATIENTS

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Background: Synchronous multiple primary malignant tumors (sMPMTs) are occasionally diagnosed during screening for a newly diagnosed malignant neoplasm. Lymphoma is one of the most common hematological malignancies, and number of lymphoma patients with sMPMTs seems to grow as the population ages. Since the standard chemotherapy for lymphoma takes a few months, treatment strategy sometimes comes to an issue. An important clinical question is how to handle sMPMTs in the treatment of lymphoma, we investigated prognostic significance of sMPMTs and suitable treatment strategy for a newly diagnosed lymphoma with sMPMTs.

Methods: We retrospectively analyzed patients with malignant lymphoma newly diagnosed between 2009 and 2015. The definition of sMPMTs was patients who were also diagnosed as a solid tumor within 6 months of the diagnosis of lymphoma. Therapeutic strategy was according to physician’s choice. Impact of sMPMTs on treatment outcome of lymphoma was analyzed. Also, relation between treatment of lymphoma and concomitant solid tumors was closely analyzed.

Results: Total of 505 lymphoma patients was included. Median age was 69 (range20-99). The most common diagnosis was diffuse large B-cell lymphoma (63%), and patients with aggressive lymphoma accounted for 77% (391/505). High risk disease, which was defined as international prognostic score 3 or higher, accounted for 36% (184/505). sMPMTs were identified in 16 patients (3%). There was no difference of distribution between patients with and without sMPMTs regarding age, grade of lymphoma, and disease risk. The overall survival (OS) and disease-free survival (DFS) were not significantly different between the two groups (with sMPMTs: 53% and 47% vs without sMPMTs: 77% and 61% at 3 years, P=0.20 and P=0.31). Cumulative incidence of lymphoma relapse was similar between the two groups (with sMPMTs 29% vs without sMPMTs 27% at 3 years, P=0.28). In multivariate analyses, age (75 years<) and disease risk (high) were identified significant risk factors for OS, and age was an only significant risk factor for DFS. Existence of sMPMTs was not a significant risk factor for either OS or DFS (OS: HR 1.29, 95%CI 0.52-3.20, P=0.58; DFS: HR 1.06, 95%CI 0.49-2.27, P=0.88). Among 16 patients with sMPMTs, half of the patients had high-risk lymphoma, and half of the solid tumors were gastric cancer. Treatment was initiated for the disease which was diagnosed earlier in all patients except one. Interval from diagnosis to the first treatment was significantly shorter in patients whose lymphoma was treated earlier (median 11 days vs 38.5 days, P=0.004). OS was not significantly different according to the sequencing of treatment (lymphoma earlier: 59% vs Solid tumor earlier: 40% at 3 years, P=0.84). In 8 of 10 patients whose lymphoma was treated earlier, treatment of lymphoma was interrupted for the treatment of the solid tumor. Interruption of treatment had no significant effect on OS ( interruption+: 29% vs without interruption: 30% at 3 years, P=0.13).

Summary/Conclusions: Existence of sMPMTs was not a significant risk factor for newly diagnosed lymphoma patients. It is important to provide adequate treatment for both lymphoma and solid tumor at physician’s discretion.
Bleeding disorders (congenital and acquired)

PB1743
GLOBAL HEMOSTATIC ASSAY AT DIFFERENT TARGET ACTIVITY OF FACTOR VIII AND FACTOR IX
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Background: Based on reports addressing hemophilia B patients bleed less common and less intensively than hemophilia A, it has been expected that the hemostatic level of factor IX (FIX) activity can be lowered to that of factor VIII (FVIII) activity.

Aims: We compared the hemostatic efficacy of the different hemostatic level of FIX and FVIII activity using global hemostatic assay.

Methods: A total of 17 severe hemophilia patients without inhibitor, aged more than 15 years were subjected; 12 hemophilia A patients and 7 hemophilia B patients. Factor concentrates were injected to reach the target activity of 60% in hemophilia A and 40% in hemophilia B which is given by Korean health insurance guideline. All patients were in non-bleeding state and kept the wash-out period of 3 days for hemophilia A and 5 days of hemophilia B. Before and on 15 minutes after injections, we conducted one-stage factor assay, thrombin generation assay (TGA), thromboelastography (TEG) and clot-wave form analysis (CWA).

Results: Median ages of hemophilia A and hemophilia B patients were 28 and 33 years old. Baseline FVIII:C and FIX:C were 0.6% and 1.8% and they rose after injection rose to 70.8% and 49.6%. The dosage of FVIII concentrates and recombinant FIX concentrates were 28.4 IU/kg and 50.7 IU/kg. In- vivo recovery (IVR) in hemophilia A and hemophilia B patients recorded 2.43% IU/kg and 0.91% IU/kg. Peak thrombin of FVIII and FIX were 451.3 mN and 376.6 mN (P=0.108, normal range, 458 mN±30). TEG index of FVIII and FIX were -1.60 and -3.77 (P=0.004, normal range, -2.23). MIN2 of CWA of FVIII and FIX were 0.62 and 0.59 (P=0.001).

Summary/Conclusions: Global hemostatic assay indicates even though IVR of FVIII and FIX are normal, less amount of FIX is insufficient to normalize hemostatic parameters in comparison with FVIII.

PB1744
THE RATE OF SUCCESSFUL IMMUNOTOLERANCE INDUCTION IN HAEMOPHILIA A BOYS TREATED WITH OCTOCOG ALFA - THE EXPERIENCE OF POLISH PAEDIATRIC HAEMOPHILIA CARE CENTRES
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Background: Development of neutralizing anti-factor VIII alloantibodies (inhibitor; INH) is the most challenging complication of haemophilia replacement therapy (HRT). It occurs in up to 30% of severe haemophilia A (HA) patients. Data published recently indicate that immunotolerance induction (ITI) is effective in 62-77% of cases.

Aims: To assess the rate of successful ITI in boys with severe HA treated with full length recombinant FVIII (octocog α) in all Polish Paediatric Haemophilia Care Centres between 2011-2016.

Methods: From 2011 to 2016 in all Polish Paediatric Haemophilia Care Centres 14/88 (15.9%) boys with severe HA on prophylaxis or on demand treatment with octocog α developed INH after 3 - 489 (median 20) exposure days (EDs). Twelve of them (85.7%) were high responders with the peak inhibitor titre (PIT) 5.88 - 716.8 (median 20.1) BU/ml. Two patients were low responders (14.3%) and had PIT 2.8 and 3.02BU/ml. All except one boys were Caucasians and had normal karyotype. All patients were under 14 years old and according to obstetrician’s estimation during the operation. Pregnancy did not cause health state deterioration in our patient and there are no clinical findings of Niemann Pick disease in newborn.

Summary/Conclusions: We presented 1 case of pregnancy in 34 year old women with Niemann Pick disease type B. Marked splenomegaly, mild thrombocytopenia and partial respiratory insufficiency existed before this pregnancy. Decisions about diagnostic assessment, platelet transfusion, splenectomy, and started prophylaxis with activated prothrombin complex concentrate (APCC). The remaining 3 patients are still on ITI. All 7 patients after successful ITI were put back on prophylaxis with octocog α.

Summary/Conclusions: 1. Octocog α is effective in induction of immunotoler- ance in severe haemophilia A boys who developed inhibitor on prophylaxis with octocog α.

Table 1. Characteristic of patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at initiation (yr)</th>
<th>PIT at initiation (BU/ml)</th>
<th>Initial activity (BU/ml)</th>
<th>Final activity (BU/ml)</th>
<th>Treatment</th>
<th>Surgery</th>
<th>Haematologist</th>
<th>Family history of haemophilia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.2</td>
<td>37</td>
<td>6.75</td>
<td>0.02</td>
<td>0.85</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OO, on demand; P, prophylaxis; CVA, central venous access; N, no; Y, yes; mth, month.
Dyslipidemia treatment was made upon data from literature and patient’s findings. Multidisciplinary approach in this setting is needed. Bleeding risk is not connected only with platelet count, but also with their function and degree of splenomegaly. Liver function can also be disturbed and can influence hemostasis. Pregnancy in our patient did not cause health state deterioration and there were no clinical findings of Niemann Pick disease in newborn.

**PB1746**

**SINGLE CENTRE FX DEFICIENCY EXPERIENCE**

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**Background:** Factor X is a vitamin K–dependent serine protease that works at the crossroads of the extrinsic and intrinsic pathways to cleave prothrombin into thrombin. Inheritance pattern of factor X deficiency is autosomal recessive, with heterozygote patients most often remaining asymptomatic or having only a mild bleeding phenotype. (1) Homozygous individuals may experience haemorrhagic symptoms, including easy bruising, haematuria, soft-tissue haemorrhages, haemarthroses, recurrent epitaxis, and menorrhagia (2) Congenital factor X deficiency is among the most rare factor disorders. We present here our experience with patients having congenital factor X deficiency.

**Aims:** We aimed to present our experience with rare FX deficiency in our centre.

**Methods:** There are currently 4 patients with factor X deficiency (F:M: 3:1) that are followed at our centre.

**Results:** First patient is 40 years old man who got his diagnosis at the age of 31 years following a gastrointestinal bleeding. He was treated with fresh frozen plasma (FFP) at that time. His FX was found:5%:0. Two years later underwent a planned tooth operation under the coverage of prothrombin complex concentrate (PCC) (Table 1). Three years after the tooth extraction he underwent an intraocular lens operation under PCC prophylaxis. No complication was observed while on PCC treatment.

**Table 1.**

<table>
<thead>
<tr>
<th>weight : 70 kg</th>
<th>PCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operation day</td>
<td>750 unit</td>
</tr>
<tr>
<td>2nd day</td>
<td>500 unit</td>
</tr>
<tr>
<td>4th day</td>
<td>500 unit</td>
</tr>
<tr>
<td>6th day</td>
<td>250 unit</td>
</tr>
</tbody>
</table>

Our second patient is a woman who was diagnosed at the age of 3 because of recurring gum bleeding. She has been treated with FFP replacement throughout her childhood and adolescence due to recurring nose and soft tissue bleeds as well as menorrhagia. She was first referred to our hospital at the age of 42 due to soft tissue bleeding. Given the lack of health insurance she mainly received FFP and tranexamic acid tablets during most of her bleeding attacks. However, PCC of 1000 unit for two days had to be used for her excessive vaginal bleeding irrespective to FFP. Her number of annual bleeding is 15-20 times in a year and most of them are gum bleeding and rarely vaginal bleeding. Third and 4th patients were referred to our centre because of prolonged the prothrombin time (PT) and the activated partial thromboplastin time (aPTT) and received the diagnosis of FX deficiency.

**Summary/Conclusions:** Bleeding phenotype differs in a wide range in patients with congenital FX deficiency. Secondary causes including amyloidosis should be excluded especially in patients receiving diagnosis at advanced ages. Usually the factor level does not correspond to the severity of the bleeding phenotype. Therefore bleeding pattern of the patients with FX deficiency should be carefully observed and considered while planning a prophylactic treatment with PCCs to prevent the risk for thrombosis and unnecessary utilisation of PCCs. FFP and PCCs replacement continue to be the source for FX in bleeding patients or in individuals requiring prophylaxis. Recently, a FX concentrate has entered the market in the USA and the European Community.

**PB1747**

**IMPROVEMENT OF THE SURVIVAL FOR LIFE-THREATENING HEMORRHAGE WITH HEMOPHILIA PATIENT**

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**Background:** In life threatening hemorrhage such as brain and abdomen, several important factors are affect for improving the survival. One tenth (223) of hemophilia patients in Korea lived in Daegu city and Kyungpook province and have been treated in our treatment center.

**Aims:** We reviewed the result of life threatening hemorrhage and our unique care of hemophilia patients for 34 years.

**Methods:** Korea Hemophilia Foundation was established in 1991. After that all factor concentrates were free to all hemophilia patients. Home treatment are easily available for rapid administration of factor concentrate of full required amount. Rapid transportation to emergency room are available for immediate operation. Hot line of mobile phone between patient and doctor for 24 hours are available for emergency care. Monthly group education has done. Prophylactic treatment was started to all who had a life threatening hemorrhage history in 1996. But HIBA permitted officially since 2011. And then recovery rate test was done for the optimal blood level for life threatening hemorrhage patient. Continuous infusion with every 2 to 4 hours reconstitution dilution fluid has been done for preserve in vitro factor activity to all surgery cases.

**Results:** Thirty five events were intracranial hemorrhage in 17, general surgery in 9 and orthopedic surgery in 9. Age distribution was 0-32 yr (mean; 24.8 yr). Severity was severe (16), moderate (7) and mild (5). Time interval between first symptom and arrival at ER were 15 min to 10 days (mean; 1.7 days). We confirmed in vivo factor activity within permissible level in all patients. All recover from hemorrhage or surgery and are healthy, but one had limb atrophy in one had mild neurologic sequelae for more than 10 years follow-up period.

**Summary/Conclusions:** Education, financial support, home and prophylactic treatment, hot-line, individual pharmacokinetics with effective blood level and fresh concentrate during continuous infusion are important factors to improve the survival of surgery case.

**PB1748**

**CAN BLEEDING SCORE AND FACTOR LEVELS DETERMINE HEMOPHILIA CARRIERS?**

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**Background:** Hemophilia A and B are X-linked recessive hemorrhagic disease. Due to this type of inheritance, males are usually affected, but girls are carriers. Factor levels are usually detected around 50% because only one chromosome is affected in carriers. Inconsistently, it has been reported that factor activity can be detected in a wide range of 22%-116% as a result of random inactivation (lyonization) of one of two X chromosomes. It is specified that factor levels may be very low due to excessive inactivation in a significant part of the hemophilia carriers, which creates a risk of bleeding in carriers.

**Aims:** In this study, we aimed to investigate the role of bleeding score and factor levels in detecting hemophilia carriers.

**Methods:** Bleeding Assessment Tool (BAT) for hereditary factor deficiencies of the International Society on Thrombosis and Haemostasis (ISTH/SSC) were applied to the mother and sisters of 32 hemophilia patients who were followed-up in Dr Behçet Uz Children’s Diseases and Surgery Training and Research Hospital. Mothers whose at least one of the other members of the family and their sons had hemophilia, mothers with more than one hemophilic son and girls whose father had hemophilia were evaluated as an obligate carrier. Sisters or mothers who do not meet the obligatory carrier criteria but whose siblings or sons are hemophilic were identified as possible carriers. Factor activity of obligate or probable carriers was studied after their informed consent was obtained.

**Results:** Thirty-two mothers and 13 sisters of hemophilia patients were included in this study. The mean age was 31.6 (4-57) years. Three of the patients were mild, 3 were moderate, 23 were severe hemophilia A; 2 were severe and 1 had moderate hemophilia B. Twelve were obligate and 33 were probable carriers. Only seven in 45 (15.5%) probably and obligate hemophilia carriers had high bleeding scores (≥4). Those with high bleeding scores, three were obligate carriers and four were probable carriers. The mean factor activity of 12 obligate and 18 probable carriers were 78.9% (20.8%>189%). Factor activities of the three obligate carriers with high bleeding scores were 77%, 80% and 98%, respectively. Factor activities of the three probable carriers with high bleeding scores were 58.8%, 69.3% and 112%, respectively. The median bleeding scores of four probable and one obligate carriers with low factor activity (<60%) were 2.8 (1-4).

**Summary/Conclusions:** Measurement of factor activity seems to be insufficient to detect hemophilia carriers. ISTH/SSC-BAT may help to determine the carriers. However, a larger study is needed to understand the diagnostic value of the BAT.

**PB1749**

**FETAL INTRACRANIAL HEMORRHAGE AS A PRESENTING FEATURE OF SEVERE CONGENITAL FACTOR VII DEFICIENCY: THE NEED FOR EARLY PROPHYLAXIS**

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**Background:** Congenital factor VII (FVII) deficiency is a rare autosomal reces-
sive bleeding disorder, with an estimated prevalence of 1:300,000. Compared to western countries, rare bleeding disorders (RBDs) are relatively commoner in Oman, owing to high rate of consanguineous marriage.

Aims: To discuss an interesting case of severe congenital factor VII deficiency and to explore the need for early prophylaxis.

Methods: Case report and retrospective data analysis of all children diagnosed with inherited coagulation factor deficiencies in Pediatric Hematology Unit, Sultan Qaboos University Hospital, Muscat, Oman from January 2009 till December 2016.

Results: We report a male full term baby, delivered by cesarean section. His older sister is a known case of severe congenital factor VII deficiency. Antenatal scans of this baby revealed two intracranial hematomas and dilated cerebral ventricles. Postnatally, the diagnosis of severe congenital factor VII deficiency was confirmed. CT scan revealed obstructive hydrocephalus at the level of aqueduct of Sylvius (Figure 1). At day 10 of life, ventriculo-peritoneal shunt has been done successfully under cover of recombinant activated factor VII replacement therapy. Afterwards, the patient has been initiated on rFVIIa prophylaxis at a dose of 30 ug/kg three times weekly. In our center, deficiencies of fibrinogen, FV, FVII, FX and FXIII were diagnosed in 22 pediatric patients (10 males and 12 females), accounting for 11.1% (22/198) of all children with inherited coagulation factor deficiencies. The age range from 1 day to 6 years and consanguinity is found in 19/22 cases (86.4%). Hypofibrinogenemia, FV and FVII deficiency are the commonest RBDs, diagnosed in 8, 6 and 5 patients respectively. As an initial presentation, intracranial hemorrhage occurred in 7/22 cases (31.8%). Three patients with FV, FVII and FXIII deficiencies suffered from global developmental delay due to severe intracranial hemorrhage. As regards management, 4 patients with severe FV deficiency and one with severe FXIII deficiency are on fresh frozen plasma (FFP) and recombinant FXIII prophylaxis respectively. Other patients receive on-demand therapy.

Figure 1.

Summary/Conclusions: Children with RBDs constitute more than one tenth of cases of hereditary coagulation factor deficiencies in our center. They have some unique features in terms of severity, clinical profile and the need for prophylaxis early in life. We recommend establishing a national/regional registry of RBDs to identify the magnitude and the peculiar genotype-phenotype correlations of such rare, yet significant disorders.

PB1750

THE ASSOCIATION OF BLOOD TYPE WITH THE NEED FOR TRANSFUSIONS IN PATIENTS WITH VENTRICULAR ASSIST DEVICES

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Background: Patients who have implantation of continuous flow ventricular assist devices (VAD) as a bridge to heart transplantation are subjected to complications secondary to pump support. The use of antiplatelets either alone or in combination with anticoagulation is necessary to avoid clot formation and pump thrombosis. However, a proportion of patients reveal an increasing risk of bleeding episodes. A possible reason of this situation could be that high shear forces lead to devastation of high molecular weight von Willebrand factor (vWF) making it functionally inactive and resulting in acquired von Willebrand disease (vWD). People with blood type O have lower baseline vWF levels and this abnormality could exacerbate the bleeding risk of patients with blood type O with VAD, resulting in more frequent bleeding episodes and need for transfusions.

Aim: The aim of current study was to investigate the possible association of blood type with acquired vWD induced by VAD, with the need for transfusions.

Methods: In this retrospective study, 17 patients who had a VAD implantation in our hospital during a six-month period were included for analysis. The investigation of underlying vWD was estimated by ristocetin-induced platelet aggregation (RIPA) using classical light transmission aggregometer.

Results: Six patients (35.3%) had left-VAD (L-VAD) implantation while the others had biventricular VAD implantation (BiVAD). The mean age was 42.41 years (SD±15.33) and 9 patients (52.9%) were male. Female patients had VAD implantation at younger age than male (p<0.001). The mean follow-up after VAD implantation was 15 months (SD±11.88). At the time of analysis, 13 patients (76.5%) were alive, 2 patients (11.8%) had died while 2 patients (11.8%) had been heart-transplanted. Eight patients (47.1%) had blood type O, 8 patients (47.1%) had blood type A and a patient (5.9%) had AB. Mean RIPA before VAD implantation was 59.3% (SD±14.76) while after VAD implantation was 47.29% (SD±15.47), whereas the decrease was no statistically related. No statistical correlation was found between RIPA among different blood types. Among patients with blood type O, the need for blood transfusions was associated with the duration of having the VAD implantation in months (p<0.001) while the need for fresh frozen plasma (FFP) transfusions was associated with RIPA before VAD implantation (p=0.016). In non-blood O type patients no statistical correlation was found with the need for transfusions with RIPA percentage or median follow-up of patients.

Summary/Conclusions: It has been shown by several studies that patients with VAD show a decrease in vWF increasing the bleeding risk. Thus the best antiplatelet treatment and/or anticoagulation that those patient needs, remains challenging. In our study, there was a decrease in mean RIPA percentage after VAD implantation and patients with blood type O had lower RIPA before implantation. However, none of these measurements was statistically significant. The blood type O patients showed an increased need for transfusions in correlation with the duration of VAD implants and an increased need for FFP in correlation with RIPA baseline. Our study has limitations due to the small population and the fact that vWF was not estimated within the different blood groups at baseline and after VAD implantation.
Bone marrow failure syndromes incl. PNH · Clinical

PB1751
ACQUIRED PURE RED CELL APLASIA ASSOCIATED WITH LYMPHOPROLIFERATIVE DISEASES IN ERYTHROPOIETIN-REFRACTORY ANEMIA PATIENTS ON DIALYSIS
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Background: Erythropoietin-refractory anemia is a serious problem and complicated cases should be ruled out in patients on dialysis. Acquired pure red cell aplasia (PRCA) may be hidden behind anemia of chronic kidney disease. Recently it was reported that PRCA patients with large granular lymphocytes frequently had STAT3 mutations (Oie ZY et al. J Hematol & Oncol 2013, Ishida F et al. Cancer sci 2014). Molecular or flow-cytometric analysis is useful for detecting a small amount of abnormal lymphocytes.

Aims: We conducted this study to determine the clinical characteristics and STAT3 mutations of patients with acquired PRCA on dialysis with lymphoproliferative diseases.

Methods: In our hospital, 4 patients were diagnosed as having acquired PRCA on dialysis with lymphoproliferative diseases after 2005. Patients were retrospectively studied for presenting feature, laboratory data, and clinical course. Surface markers of lymphocytes were examined by flow cytometric analysis, and T-cell receptor (TCR) rearrangements were examined by Southern blot analysis. Mononuclear cells were separated after obtaining written informed consent. STAT3 (Y640F and D661Y) mutations were examined by allele-specific PCR. Current study was conducted within the guidelines and with the approval of the institutional ethical committee.

Results: In spite of adequate administration of erythropoietin colony-stimulating factor, all 4 patients required blood transfusion due to erythropoietin-refractory anemia. Mean leukocyte and lymphocyte counts at diagnosis were 4650/mL (range, 3180-4850) and 1794 mL (range, 1183-2859), respectively. Two patients (Cases 1 and 2) had low percentage of CD4+ CD8+ by flow-cytometry and TCR C beta1 and gamma rearrangements by Southern blot analysis. Another patient (Case 3) had high percentage of gamma-delta T cell component (66.2%) with TCR delta rearrangement. The other patient (Case 4) had high CD16+CD56+ NK cell percentage without TCR receptor rearrangement. The surface markers of lymphocytes were examined by flow cytometric analysis, and T-cell receptor (TCR) rearrangements were examined by Southern blot analysis. Mononuclear cells were separated after obtaining written informed consent. STAT3 (Y640F and D661Y) mutations were examined by allele-specific PCR. Current study was conducted within the guidelines and with the approval of the institutional ethical committee.

Summary/Conclusions: STAT3 mutations were examined by allele-specific PCR. Current study was conducted within the guidelines and with the approval of the institutional ethical committee.

PB1753
REACTIVATION OF HEPATITIS B VIRUS INFECTION IN APLASTIC ANEMIA PATIENTS
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Background: There is little data about the influence of infection of HBV on the therapy of aplastic anemia.

Aims: This article is aimed at assessing the HBV reactivation risk in HBSAg-positive or HBSAg-negative, antithetapies B core antigen antibody (anti-HBc) - positive patients with AA receiving CsA and/or ATG.

Methods: We analysis the clinical data of 60 AA patients with HBV infection out of 201 cases of AA from our center at AA diagnosis during the recent 3 years, and laboratory test data such as levels of liver enzyme, HBV DNA in serum, HBsAg anti-HBs and anti-HBc were monitored. Entecavir (ETV) or lamivudine (LAM): was started when HBV reactivation (defined as detectable HBV DNA) was encountered or as an antiviral prophylaxis regimen for some patients with positive anti-HBc. The patients were followed up for 2 years after the lymphoma onset. The other two patients are still alive without blood transfusion for 6 and 7 years.

Results: Among 60(29.8%) AA patients, 12 were chronically infected (HBSAg positive) and 48 were previously exposed (HBSAg negative/anti-HBc positive). 5 patients (8.33%) who were HBSAg positive and not given any prophylactic antiviral therapy suffered HBV reactivation. 7 patients who were HBSAg positive but given were found no HBV reactivation during the follow-up. All the 48 patients with negative HBsAg and positive anti-HBc were found no HBV reactivation during the follow-up.

Summary/Conclusions: Antiviral prophylaxis should be recommended for HBSAg-positive patients who will receive IST with AA as they had high rate (41.6%) of HBV reactivation. HBV infection were found no influence to the clinic course in AA and antiviral therapy had no influence to the effect of IST.

PB1754
MULTICENTER RESULTS OF SCHWACHMAN-DIAMOND SYMPOD PATIENTS
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Background: Schwachman-Diamond syndrome (SDS) is an autosomal recessively inherited disease characterized with neutropenia, exocrine pancreas insufficiency, failure to thrive and skeletal abnormalities. In approximately 90% of the patients, the molecular defect is related to SBDS gene mutations. The classical triad is present in one-forth of the patients and a high degree of suspicion is required in order to make the diagnosis. In this study, molecular work-up to patients with suspected SDS were made and the clinical and laboratory findings that predict the SDS diagnosis were investigated.

Aims: The aim of the study was to find out the predictive clinical and laboratory characteristics of SDS patients. To do so, the patients were sent to Hacettepe Inherited Bone Marrow Failure Center for molecular work-up between June 2013 and August 2016 were evaluated with clinical and laboratory data obtained from a standardized patient registry form.
Results: Molecular work-up was performed in 20 patients referred to our center with a suspected diagnosis of SDS. Of these 20 patients (12 girls), 4 (20%) (3 boys) were found to have mutation in SBDS gene. The median age of these patients was 3.2 years (1-18). Of the 4 patients with genetically verified SDS, 1 (25%) had history of chronic diarrhea and pancreas atrophy detected in ultrasonography of that patient. Another patient (25%) with SDS had skin ulcers and one (25%) of the patients had failure to thrive. Three patients (75%) had anemia associated to neutropenia, and 1 patient (25%) had pancytopenia at presentation. On the other hand, the patients who were referred with a suspicion of SDS but was found to have no mutation, 43% had neutropenia, 25% had bictopenia, 10% had pancytopenia. The patients of the latter group had failure to thrive in 25% of the patients and chronic or persistent diarrhea was present in 25% of this group. There was no statistically significant difference in the clinical and laboratory parameters of the genetically verified SDS patients and patients without SDS but was referred with SDS suspicion.

Summary/Conclusions: Although, there was no statistically significant difference in the clinical and laboratory parameters of the genetically verified SDS patients and patients without SDS but was referred with SDS suspicion, this might be attributed to the small sample sizes. Compatible with the previous literature data, SDS is a cryptic disorder and the classical triad is not commonly fulfilled in most of the patients. On the other hand, failure to thrive/growth retardation was three times more common in patients with SDS. Thus, in patients neutropenia, accompanying failure to thrive/growth retardation might be an indicative to make molecular work-up for SDS. Additionally, not only neutropenia, but bictopenia or pancytopenia might be the hematological presentational findings of SDS.

PB1755
PAROXYSMAL NOCTURNAL HEMOGLOBINURIA AND APLASTIC ANAEMIA - DATA FROM THE SPANISH PNH REGISTRY
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Background: Aplastic anemia (AA) and Paroxysmal Nocturnal Hemoglobinuria (PNH) are included, together with other pathologies, within the bone marrow failure syndromes (BMFS). In the present paper, these clinical entities could be understood as independent pathologies, due to the extremely frequent evolution among them and with other BMFS, along with the development of new clones in the context of haematopoietic stem cells’s kinetics.

Aims: The aims of this study were analyzing and comparing the behaviour of patients with PNH who suffered from PNH with pancytopenia with respect to that of patients who were initially diagnosed of AA and who later developed a PNH clone.

Methods: A clinical form was elaborated and distributed among the investigators of the PNH Spanish Registry. Clinical, laboratory and treatment data of the patient were asked. Soon after, a descriptive analysis of the data was performed.

Results: 34 patients were recruited and analyzed (12 women and 22 men). Their age interval ranged from 2 to 87 years, and all of the patients suffered from either PNH with pancytopenia and/or AA with a developing PNH clone. The average age at the time of initial diagnosis was 28.5 years old (4m-72y). The average age at the time of diagnosis of patients with PNH with pancytopenia (1), moderate AA (16), severe AA (10), very severe AA (7). 15 patients presented a PNH clone in their granulocytes and/or monocytes at the time of diagnosis, being 24% the average of such clone (0-88%) and less than 2% in 7 patients. All of these patients who showed hemolitic signs at diagnosis presented clones >20%. The time of the clone’s development in patients without PNH was 2 to 11 years (3-16). Treatment response with eculizumab in 2 patients just after resolution. Patients with AA received an average of 3 treatments, mostly cyclosporine with/without ATG (anti-thymocyte globulin), 10 patients underwent HCT (7 allogeneic HCT and 3 matched unrelated-donor HCT). 14 patients received eculizumab, being 88.2% the average size of the PNH clone at diagnosis (85-99%). Treatment response with eculizumab was total in 11 cases and partial in 3. The following complications were observed: cholelithiasis (3), renal failure (6; 50% secondary to treatment), iron overload that required chelation therapy (3), transient aplastic crisis due to parvovirus B19 (1), HCV infection (1), thrombosis (6). The patients started anticoagulant treatment (15 patients) with no evidence of further thrombosis once the treatment was initiated. 28 patients remain alive (26 of them with very good quality of life), 3 of them died due to HCT-complications and follow-up was lost in the 3 remaining cases.

Summary/Conclusions: Clonal evolution in AA is frequently associated with the development of a PNH clone at the time of diagnosis, throughout the pathology’ natural course or even after disease’s resolution. The development of such clone has been related to better prognosis in AA right after the immunosuppressive therapy (IST). Our experience demonstrated the presence of hemolisis in at least half of the cases, making it necessary in these patients treatment with eculizumab, generally obtaining a very good response.

PB1756
AUTOIMMUNE CYTOPENIAS IN PRIMARY IMMUNODEFICIENCY DISEASES: SINGLE CENTER EXPERIENCE
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Aims: Primary immunodeficiency diseases (PIDS) are associated with hematologic complications such autoimmune hemolytic anemia (AIHA) and thrombocytopenia (ITP). The most common autoimmune cytopenia is ITP. Although ITP is observed in 7.6% of patients with PID, AIHA is seen at 4.8%. Also, we aimed to present the patients who had autoimmune cytopenias and PID.

Methods: Fifty six PID patients who were followed at the Pediatric Immunology Department of Erciyes University Medical Faculty (they were analyzed genetically) were evaluated retrospectively. Autoimmune cytopenias such as ITP and AIHA were detected in 9 (9.16, 0.7) of the patients (combined immunodeficiency:4 patients, common variable immunodeficiency: 2 patients, hyper immunoglobulin E syndrome:1 patient, X-linked lymphoproliferative :1 patient, chronic granulomatous disease:1 patient). ITP was detected in 8 of 9 patients and AIHA was also detected in 6 patients. In four patients (LRBA deficiency: 2 patients, hyper IgE syndrome:1 patient and OSD:1 patient), both ITP and AIHA were observed. Immunosuppressive therapy with steroid, cyclosporine, mycophenolate mofetil and intravenous immunoglobulin were given to all patients. Bone marrow transplantation was performed to the four patients. However, five patients died because of immunodeficiency.

Results: There is a paradoxical situation between PID and autoimmunity. The reduction of central and peripheral tolerance is held responsible for autoimmunity in PID.

Summary/Conclusions: As a conclusion, we wanted to point out autoimmune cytopenias in patients with PID and the role of immunodeficiency in the approach for treatment.

PB1757
HEAVY METAL LEVELS IN PATIENTS WITH FANCONI APLASTIC ANEMIA
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Background: Fanconi aplastic anemia (FAA) is a rare, autosomal recessively inherited bone marrow failure syndrome. Variants of congenital anomalies may accompany disease and various complications including malignancy and endocrinopathies may develop during the course.

Aims: Heavy metal levels in patients with different chronic disorders have been investigated, however, up to our knowledge there is no literature data on the heavy metal levels in patients with FAA. Heavy metal levels in patients with different chronic disorders have been investigated, however, up to our knowledge there is no literature data on the heavy metal levels in patients with FAA.

Methods: Study was performed between July 2015 and April 2016 among patients with FAA and the results were compared with age and gender matched healthy control group.

Plasma copper (Cu), zinc (Zn), lead (Pb), chromium (Cr), cobalt (Co), selenium (Se) levels were measured in patients with FAA.

Results: Total of 17 patients with FAA were included in the study. Median age was 9 years (1-30), female to male ratio was 8/9. One patient had undergone stem cell transplantation, four patients were transfusion dependent. When we compared patients with FAA and age/sex matched healthy group (16 volunteers) Cr and Cu levels were higher and Se level was lower in FAA group significantly (Table 1). However, all patients had chromium level within normal range, two patients with FAA and two volunteers had copper levels higher than the normal ranges (Table 2).

Table 1. Heavy metal levels in patients and control group.

POPULATION | Copper (mg/L) | Zinc (mg/L) | Selenium (mcg/L) | Lead (mcg/L) | Chromium (mcg/L)
--- | --- | --- | --- | --- | ---
Control | 1.9 (1.5 - 2.3) | 97.8 (93.7 - 101.9) | 0.13 (0.11 - 0.16) | 0.2 (0.1 - 0.3) | 0.2 (0.15 - 0.24)
FAA | 2.5 (2.2 - 2.8) | 102.2 (98.2 - 112.1) | 0.11 (0.08 - 0.16) | 0.2 (0.1 - 0.3) | 0.2 (0.15 - 0.24)

FAA; Fanconi aplastic anemia.
Table 2. Classified heavy metal level in patients and controls.

<table>
<thead>
<tr>
<th>Metal</th>
<th>FAA</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromium</td>
<td>Low</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>20</td>
</tr>
<tr>
<td>Cobalt</td>
<td>Low</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>20</td>
</tr>
<tr>
<td>Copper</td>
<td>Low</td>
<td>17</td>
</tr>
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<td></td>
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<td>Iron</td>
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<td>Normal</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>20</td>
</tr>
<tr>
<td>Zine</td>
<td>Low</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>20</td>
</tr>
</tbody>
</table>

FAA: Fanconi aplastic anemia.

Summary/Conclusions: In our study we found chromium and cobalt levels higher in patients with FAA than control group. In-vitro studies have revealed that FAA cells are more sensitive to chromium toxicity. With larger number of patients chromium level and clinical association should be investigated in further studies. Lower Se level in patients with FAA may be related with oxidative stress in these patients.

PB1758

CLINICAL IMPACT OF AGE AND COMORBIDITY IN PNH PATIENTS

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Background: PNH is an ultra-rare disorder affecting mainly young adults, but can be diagnosed in geriatric population. Comorbidity is more prevalent in geriatric population and can either hamper diagnostic evaluation or increase the complexity of PNH patient care.

Aims: To identify geriatric-age PNH in Spanish PNH registry. To study the clinical characteristics at diagnosis and evolution of geriatric-age PNH and compare them to non-geriatric PNH population. To analyse the impact of both age and prognosis in the PNH setting. To evaluate the use of eculizumab in geriatric age patients.

Methods: In a multicentric retrospective study, Cumulative Illness Rating Scale for Geriatrics (CIRS-G) and clinical and biological variables have been collected from a Spanish PNH Group patient cohort. Statistical analysis was performed using GraphPad Prism v5 (La Jolla, CA).

Results: 44 patients from 11 centres in Spain have been included up to date. 8 patients (17.8%) were diagnosed in geriatric age (equal or older than 65 years) (Age range for the complete cohort: 17-83 years) and 9 patients presented with high comorbidity, arbitrary defined as CIRS-G score >10. (Range for the complete cohort: 3-13) A weak comorbidity were poorly correlated (p=0.0187, R-square 0.15) No differences in clinical presentation (Classic, PNH in the setting of another bone marrow failure syndrome or Subclinical PNH or high disease activity) when stratifying by age or comorbidity were observed. 4 patients had a concomitant myeloid clonal disorder (3 myelodysplastic syndrome and 1 myeloproliferative neoplasm, 3 of them (75%) in geriatric age. Median follow up was 7.2 years. Both age equal or older than 65 years and CIRS-G >10 were associated to poorer overall survival (HR: 0.0134 and 0.045 & p=0.0015 and 0.0103 respectively). Regarding PNH with high disease activity, 16 patients were identified, 4 of them in geriatric age. In 2 of them (50%), Eculizumab was used, which contrasts with eculizumab use in younger patients (78.6% in the same indication) Regarding comorbidity impact on eculizumab therapy outcome, 2 patients had CIRS-G score >10 and had similar overall survival as patients with lower comorbidity in this cohort.

Summary/Conclusions: Age and comorbidity are associated with poorer overall survival in PNH. Older age and comorbidity may not preclude the use of effective treatment in PNH patients, including those with high disease activity. Prospective evaluation of comorbidity in PNH patients, regardless of age is warranted.
Chronic lymphocytic leukemia and related disorders - Biology

PB1760
LDH AS PREDICTIVE PARAMETER IN TREATMENT-NAÏVE PATIENTS WITH TRISOMY 12 CHRONIC LYMPHOCYTIC LEUKEMIA


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Background: Patients affected by chronic lymphocytic leukemia (CLL) that have trisomy 12 (+12) on FISH analysis have unique clinical and biological features. In a prior analysis (Autore F, ASH 2016) of 487 patients with +12 compared to 816 patients with negative FISH, patients with +12 had a significantly higher prevalence of elevated LDH, β-2-microglobulin, ZAP70 positivity, CD38 positivity, CD49d positivity and unmutated IGHV as compared to patients with negative FISH. They also showed shorter progression free survival (PFS), treatment free survival (TFS) and overall survival (OS) (Figure 1A). Aims: To identify clinical and laboratory features that predict disease progression, time to treatment and survival in treatment-naïve patients with +12 CLL. Methods: This study included 487 treatment-naïve patients with +12 CLL from 16 academic centres, diagnosed between January 2000 and July 2016. A cohort of 250 patients with +12 CLL followed at a single US institution was used as external validation. Data were summarized as medians and 25th and 75th percentiles. Chi-square test or Fisher’s exact test were used to compare categorical variables, while Wilcoxon-Mann-Whitney-Test was applied for continuous variables. The survival analysis was based on the Kaplan-Meier method and the log-rank test was used to compare survival curves. A Cox model was used for multivariate analysis of the impact of different factors on survival. P values lower than 0.05 were considered statistically significant (STATA 12.0) and reported as two-sided. We analysed also CLL-specific survival considering events deaths due to the haematological disease and and 184 within normal range. Patients with high LDH levels showed shorter PFS (30 months vs 65 months, p<0.001; Figure 1A), TFS (33 months vs 69 months, p<0.001; Figure 1B), OS (131 months vs 181 months, p<0.001; Figure 1C) and CLL-specific survival with a rate of attributable mortality of 29% vs 11% (p<0.001). In the validation cohort, 104 patients had high LDH levels and 145 patients had normal LDH levels; factors significantly associated with PFS and TFS on univariate analysis were LDH, β-2-microglobulin, Rai stage and ZAP70; LDH, β-2-microglobulin and age associated with OS. On multivariate analysis high LDH was the sole parameter significantly associated with all shorter outcomes, along with elevated β-2-microglobulin, which associated with shorter OS. Summary/Conclusions: Our study on 487 patients with +12 CLL and the analysis on 250 patients of the validation cohort showed that patients with +12 and elevated LDH have shorter PFS, TFS, OS and CLL-specific survival.

PB1761
THE PERCENTAGE OF CELLS WITH ABNORMALITIES IN FISH STUDIES CONFERS PROGNOSTIC INFORMATION IN CLL PATIENTS

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Background: Genomic aberrations detected by FISH have become one of the most important and widely used prognostic factor for chronic lymphocytic leukemia (CLL) patients. In addition several publications have described that patients with a higher percentage of abnormal nuclei have a worse outcome. Aims: To analyze the effect of the percentage of abnormal nuclei detected by FISH (13q deletion (13q-), 11q deletion (11q-), 17p deletion (17p-) and trisomy 12 (+12)) in overall survival (OS) and time to first treatment (TTFT). Methods: We studied a non-selected cohort of 650 consecutive CLL cases from a local database with a median follow up time of 50 months (0-346). The cut-off point for the percentage of abnormal nuclei for each alteration was detected by dividing the variable into deciles, and selecting the most efficient cut-point, and based on previous publications. Results: FISH detected aberrations in 85% of the cases (442/500). The most frequent abnormality was 13q-, observed in 302 patients (47%), but as a sole alteration in 212 cases, followed by +12 (106 patients, 16%), 11q- (83 patients, 13%), and 17p- (33 patients, 5%). As expected, the group of patients with 13q- as a sole abnormality was the one with the better OS (195 months) followed by the group of patients with normal FISH (160 months), +12 (124 months), 11q- (56 months) and 17p- (46 months), consistent with the Dohner hierarchical classification (Döhner H et al. NEJM 2000). Similar results were observed in TTFT: 13q- as sole abnormality (106 months), normal FISH (112 months), +12 (29 months), 11q- (10 months), 17p- (10 months). The best predictive cut-off point that divided patients according to its prognosis was different for each alteration. We confirmed that a high percentage of cells carrying the deletion is associated with a significantly worse TTFT in cases with 17p, 13q, and 11q deletions, and a significantly shorter OS in cases with 17p deletion. We observed a similar trend for OS in cases with 13q and 11q deletions, probably not significant because of the low number of patients included, compared to previous studies. We observed the same trend in patients with +12. The Table 1 summarizes these findings. Probably with a higher number of cases and a longer follow up, it could have also been possible to reach statistically significant differences in the subgroups in which it was not objected.

<table>
<thead>
<tr>
<th>FISH abnormality</th>
<th>Number of cases</th>
<th>Overall survival (months)</th>
<th>P</th>
<th>Time to first treatment (months)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;75% abnormality</td>
<td>215</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>75% - 85%</td>
<td>194</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;85% abnormality</td>
<td>151</td>
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</table>

Summary/Conclusions: Not only the type of cytogenetic abnormality but also the percentage of abnormal nuclei detected by FISH are important factors in the prognosis of CLL patients.

Figure 1.
Results: Parameters associated with shorter PFS, TFS, OS and CLL-specific survival on univariate analysis were IGHV, LDH, β-2-microglobulin and Rai stage; age, ZAP70 and CD38 associated with OS only; on multivariate analysis, high LDH and unmutated IGHV remained significantly with shorter PFS, TFS, OS and CLL-specific survival, higher Rai stage with shorter PFS and elevated β-2-microglobulin with shorter OS. Considering interestingly the association of a simple and new laboratory parameter such as LDH to the outcomes, confirmed on multivariate analyses for PFS (hazard ratio [HR] 1.55, 95% confidence interval [CI] 1.1-2.7; p=0.034) and CLL-specific survival (HR 3.86, 95% CI 2.0-7.5; p<0.001), we divided our +12 CLL cohort according to LDH levels available at diagnosis: 103 patients showed LDH levels above the normal limit and
METHYLATION STATUS OF RAD21 GENE IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic Lymphocytic Leukemia (CLL) pathogenetic mechanisms have not been fully elucidated yet. However, genetic and epigenetic alterations seem to be involved in the pathogenesis and extensive clinical heterogeneity of the disease. DNA methylation in Cpg sites of a gene promoter, which may affect the chromatin structure as well as gene transcriptional activity, is a crucial epigenetic modification in CLL. RAD21 gene is involved in DNA repair and its encoded product acts as basic subunit of the Cohesin protein complex that regulates the cohesion and proper separation of sister chromatids during mitosis or meiosis.

Aims: We investigated the methylation status of RAD21 gene promoter and its possible implication in CLL pathogenesis and the formation of CLL cytogenetic aberrations.

Methods: The study included 105 CLL patients and 17 healthy donors (controls). Total genomic DNA extraction was performed from bone marrow or peripheral blood samples of all patients and controls. Methylation analysis of RAD21 gene promoter was carried out using the new technology of MethylScren™ in the CXF96Biorad Real-Time PCR system. For this specific purpose, we used EpTect Methyl II PCR Assay which enables us to calculate the methylation and unmethylation fraction after simultaneous digestions with specific restriction enzymes. The genotypic analysis was performed on unstimulated cells with Cpg-oligonucleotide DSP-30 bone marrow cells of CLL patients. FISH analysis was carried out using the commercial CLL set probes for detection of the most common abnormalities of the disease including deletions of 17p13 (TP53), 11q22.3 (ATM) and 13q14.33/34.3 (D13S319/13q34) regions and trisomy 12 (CEP 12).

Results: Among the 105 CLL patients, 21 patients exhibited a normal karyotype also confirmed by FISH and 84 patients showed chromosome aberrations detected by karyotypic or/FISH analysis. Methylation study was successful in all healthy donors and in 101 out of 105 CLL patients. All healthy donors had non-methylated RAD21 gene promoter. On the contrary, 25.74% (26/101) of CLL patients carried >10% cells with methylated Cpg islands in RAD21 promoter, which was significantly increased compared to controls (p=0.039, χ²=24.25, df=1). RAD21 methylated cell fraction varied among patients. More specifically, 9% of patients (10/101) showed 11-50% methylation rate, 10.89% (11/101) 51-95% and 4.95% (5/101) showed high methylation rate score, >90% of the analyzed cells. Stratification of patients according to cytogentic findings showed that the promoter of RAD21 was methylated in 28.57% of patients (6/21) with normal karyotypes and 25% of patients (20/80) with abnormal karyotypes. In detail, methylation in RAD21 promoter was present in 33.33% of patients with trisomy 12 (16/48), in 33.33% (4/12) with abn(8), in 31.25% (5/16) with -17(del17p), in 27.78% (5/18) with trisomy 12, in 25.81% (8/31) with del(13q) in 20% (2/10) with del(6q) and in 12.5% (2/16) with del(11q). Based on karyotypic complexity, RAD21 promoter was methylated in 18.18% (4/22) of patients with a single chromosome aberration, 26.09% (6/23) with two chromosome aberrations and 25.71% (9/35) of patients with complex karyotype (>3 aberrations).

Summary/Conclusions: Methylation of RAD21 gene promoter, which leads to transcriptional inactivation and consequently inhibition of RAD21 expression, seems to be implicated in CLL pathogenesis and the formation of specific chromosomal aberrations. Methylation of the epigenetic landscape of CLL may help in the design of new targeted therapeutic agents.

ROLE OF KEAP1-NRF2 PATHWAY GENETIC VARIABILITY IN THE SUSCEPTIBILITY AND PROGNOSIS OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic lymphocytic leukemia (CLL) is a heterogeneous disease, with variable clinical presentation and evolution. Two major subtypes can be distinguished, mutated (M) and unmutated (UM), characterized respectively by a high or low number of somatic hypermutations in the variable region of immunoglobulin genes and different outcome. Cytotegnic and FISH (fluorescence in situ hybridization) studies have proved to be important tools in the biologic characterization of this disease, allowing the identification of distinct risk groups. Genomic instability involves a process prone to the accumulation of chromosome alteration in somatic cells and is a major driving force of tumorigenesis. The analysis of chromosome aberrations (CA) and microneucleus (MN) variants by different forms to evaluate genomic instability.

Aims: In this study, we have analyzed the basal frequency of CA and MN in untreated CLL patients. Results were evaluated in relation to different prognostic factors.

Methods: A total of 67 untreated CLL patients (36 males; mean age: 66.6 years; range: 42-83 years; Rai stage: I: 27%; II: 59%; III-IV: 14%), and 6 normal controls, were studied. Chromosome analysis was performed on peripheral blood lymphocytes cultures. For each patient, CAs were evaluated on 50 cells stained with 10% Giemsa and the MN frequency was assessed on 250 interphase nuclei. FISH analysis was performed using the CLL panel according to the manufacturer’s protocol. GSH (Glutathione) was used for analysis in 139.4±10.2 months.

Results: The results showed that patients with the GG genotype (NFE2L2 -17/A -17) were at higher risk of developing CLL [Odds ratio (OR): 2.032; 95% confidence interval (CI): 1.234-3.51; P=0.004]. In the same way, the genotypic profile (GP) GG / CC (NFE2L2 / KEAP1) is a risk factor (OR: 2.186; 95% CI: 1.273-3.744; p=0.003) for the development of CLL while the AA / CC profile constitutes a protective factor (OR: 0.634; 95% CI: 0.407-0.984, p=0.037). In contrast, patients with genotype AG (NFE2L2) and/or CC (KEAP1) had a higher rate of complete response to rituximab therapy regimens (NFE2L2 AG: OR 1.6, 95% CI: 1.065-2.393; p=0.037; KEAP1 AG / CC: OR 1.2, 95% CI 1.041-3.477, p=0.045, NFE2L2 / KEAP1 AG / CC: OR 1.9, 95% CI: 1.843-4.485, p=0.017) and with fludarabine (NFE2L2 / KEAP1 AG / CC: OR 1.5, 95% CI: 1.199-3.887, p=0.026). Finally, the overall survival of CL patients appears to be influenced by the genotypic profile of NFE2L2 / KEAP1 [GP AG / AC patients have a lower mean survival (198.0±13.6 months) than patients with other GPs (139.4±10.2 months, p=0.037)], while progression-free survival seems to be influenced by the KEAP1 genotype [patients with CC genotype have a longer mean survival (198.0±13.6 months) than patients with AA and AC genotypes (85.3±13.4 months, p=0.023)].

Summary/Conclusions: This study suggest that genetic polymorphisms in NFE2L2 and KEAP1 genes might be risk factors for CLL development and may constitute novel genetic markers for therapy response (namely regimes with rituximab and fludarabine) as well as prognostic markers, by influencing overall survival and progression free survival in CLL patients.

The authors declare no conflicts of interest.
Results: An increased number of CAs, including chromatid breaks and dicentrics, in CLL patients (6.59±5.3%) compared to controls (0.25±0.04%) (p=0.021) was observed. A tendency to increased CA frequency in cases with abnormal (8.18±6.1%) compared to normal karyotypes (5.67±4.4%) (p=0.08) was also found. The analysis taking into account FISH risk groups showed a higher frequency of CA in patients with deletions 11q22 and/or 17p13 associated to poor outcome (8.54±4.9%), than those with no alterations or 13q14 deletion related to a better outcome (5.64±3.9%) and cases with +12 with an intermediate prognosis (4.54±3.5%). By MN analysis, an increased frequency in CLL patients (2.8±1.5%) compared to controls (0.67±0.3%) (p=0.0001) was found. Patients with +12 presented the highest percentage of MN compared to the other two groups (+13-fold), indicating the aneugenic effect of this alteration. The evaluation according to the iGHV mutational status showed similar frequencies for CAs and MN in M-CLL (6.2±5.2% and 2.6±2.4%, respectively) and UM-CLL (6.2±5.8% and 2.7±1.3%, respectively). No association between CA and MN frequencies and clinical parameters was found.

Summary/Conclusions: Our results confirm the presence of basal genomic instability in untreated CLL patients as measured by both CA and MN techniques. To our knowledge, this is the first analysis of these parameters taking into account prognostic factors of the disease. Cases with deletions 11q22 and/or 17p13 had the highest value of CA and those with +12 showed the highest frequency of MN, reflecting different mechanism of DNA damage.

PB1765

B CELLS RESISTANT TO CD20 MONOCLONAL ANTIBODIES DISPLAY SPECIFIC ALTERATIONS IN GENE EXPRESSION PROFILE

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Background: CD20 monoclonal antibodies (mAbs) are a standard of care for B-lymphoid malignancies. Yet, their clinical efficacy is quite variable and many patients relapse, while their malignant cells express very low density of CD20 on the cell surface. In spite of being used for 20 years as a therapy target, little is known about the biology and regulation of CD20 inside the cell.

Aims: The aim of this proposal was to investigate the intracellular mechanisms regulating expression of CD20 antigen.

Methods: Diverse cell and molecular biology techniques were used, including flow cytometry analysis, real-time PCR and RNA sequencing.

Results: We show that treatment of B cells with different CD20 mAbs initiates a signaling cascade within the cells that is partially distinct from classical B-cell receptor signaling machinery and does not involve BCR proximal proteins. Importantly, it results in a prompt downregulation of CD20 expression. Through chromatin exposure to gradually increasing doses of monoclonal antibodies, we have generated cell lines that are resistant to additional treatment with mAb. Notably, these cells are resistant also to any other of the available anti-CD20 antibodies even at very high concentrations as shown by dose-response experiments. This resistance is sustained for long period and maintained even upon multiple rounds of cell passages. We could also detect in these cells a regulated CD20 protein from the cell surface and that this effect was not just due to its internalization. Instead, we detected a defect in CD20 transcription as measured by quantitative real-time PCR. Flow cytometry analysis of other surface markers showed a strong upregulation of CD55 and CD59, known inhibitors of complement activation. The combination of CD20 loss together with the increase of CD55 and CD59 is responsible for the complete resistance to the mAbs. We have then analyzed changes in overall gene expressions by performing RNA sequencing and quantitative real-time PCR. We have identified several interesting genes whose expression was altered in our resistant cells when compared to their sensitive counterparts. Among the most interesting hits was a strong downregulation of the transcription factor NFκB, which was expressed more than 10-fold lower in the rituximab or ofatumumab resistant cells. We could confirm this result in multiple independent experiments. We have postulated that anti-CD20-triggered signaling results in the inactivation of NFκB, thus blocking the block in CD20 transcription. In order to test this hypothesis, we have treated the cells with phosphor-est PMA, which nonspecifically activates NFκB. Indeed, cells treated with PMA managed to rapidly upregulate CD20 on their cell surface.

Summary/Conclusions: In summary, CD20 triggering by therapeutic mAbs induces marked downregulation of CD20 transcription and intracellular changes that result in downmodulation of CD20 expression. Further analysis of detailed intracellular mechanisms regulating CD20 is warranted in order to propose novel interrogation nodes that might modulate CD20 surface density and thereby enhance the therapeutic potential of CD20 monoclonal antibodies.

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PB1766

DIFFERENTIAL EXPRESSION PATTERNS OF CHEMOKINE RECEPTORS IN CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background: Chemokines and their receptors are involved in the regulation of cell recruitment, survival, proliferation, and trafficking, all these processes crucial in the pathogenesis of chronic lymphocytic leukemia (CLL). Comprehensive profiling of chemokine receptors in CLL and its subgroups according to prognostic relevance is missing.

Aims: To characterize the chemokine expression pattern in CLL patients and subgroups according to clinical course and cytogenetic aberrations.

Methods: We studied the gene expression pattern of 16 canonical and 4 atypical chemokine receptors in peripheral blood mononuclear cells (PBMC) of CLL patients (n=88) and healthy subjects (n=34) by using SmartChip quantitative RT-PCR (WaferGen Bio-systems). The expression of CXCR3, CXCR4, CXCX6, CXCX7, and CCR7 was confirmed by flow cytometry.

Results: Among deregulated receptors, 5 receptors (CCR7, CCR10, CXCR3, CXCR4, CXCR5) were up-regulated and 9 receptors (CCR2-CRR6, CCR8, CCR9, CXCR1, CXCR2) were down-regulated in CLL; the latter did not differ between CLL and controls (P>0.05). In patients with del(17p) associated with a poor prognosis, we observed higher mRNA levels of CXCR6, CCR7 and CCR10 comparing to del(13q). On protein level, the percentage of neoplastic B cells positive for CXCR4, CXCR5, and CCR7 was higher and percentage of CCRX7 lower than on normal B cells (P<0.05). In patients with CLL a marked increase in MFI of CXCR4 (P<0.001) and CCR7 (P<0.001) on CLL cells was detected compared to healthy subjects.

Summary/Conclusions: Our results provide a complete picture of expression patterns of chemokine receptors in PBMC of CLL patients and prognostically relevant subgroups. Further studies are needed to clarify how chemokine receptor network affects neoplastic development and progression.

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PB1767

RESIDUAL SERUM CONCENTRATIONS OF RITUXIMAB ARE ASSOCIATED WITH RELAPSE RISK IN CHRONIC LYMPHOCYTOPITIC LEUKAEMIA

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Background: Rituximab is an anti-CD20 chimeric monoclonal antibody approved in first-line treatment of patients with chronic lymphocytic leukemia (CLL), in association with chemotherapy. Rituximab displays a time-dependent pharmacokinetic with a high variability between patients that is primarily related to target mediated elimination.

Aims: Rituximab pharmacokinetics has been associated with clinical response but there is no data on its association with patients’ evolution after immunochemotherapy, which is the aim of the present study.

Methods: Residual serum concentrations of rituximab were determined by an enzyme-linked immunosorbent assay (ELISA) for 35 CLL patients before each infusion, administrated every 28 days at T0, T1, T2, T3, T4, T5. Response and relapse criteria were evaluated according to the International Workshop on Chronic Lymphocytic Leukemia guidelines.

Results: Patients were assigned to two groups related to time to relapse. The first group (n=7), had an early relapse in less than 3 years, the second group (n=28) had a relapse in more than 3 years. A lower residual serum rituximab concentration was observed in patients with an early relapse and statistical significance was reached for the values obtained after the 3rd cycle (T3) (p=0.02). Concerning the area under the curve (AUC), the difference was significant across all the infusions (T0 vs T4: p=0.05; T4 vs T5: p<0.05). No difference was observed when comparing the area under the curve (T0 vs T4: p=0.02; T4 vs T5: p=0.02). Additionally, the residual rituximab serum concentration between T2 and T5, superior at 70µg/mL, is associated with a long response time, with a sensibility of 100% and a specificity of 52%. Low residual serum rituximab concentrations in the early relapse group were associated with a worse outcome and a higher 5-year progression-free survival (PFS) with the chemotherapy rituximab-bendamustine than rituximab-fludarabine-cyclophosphamide. On the other hand, there was no association with age, sex, cytogenetics, tumour burden or with FCGR3A-158VF polymorphism. Furthermore, we found a correlation between the residual rituximab serum concentration in patients with CLL has an impact on clinical evolution after treatment. This study provides data that sustains the need of rituximab serum concentration adaptation in certain CLL patients, in order to reduce relapse risk.
PB1768
ACTIVITY OF THE CD19 ANTIBODY MOR208 IN COMBINATION WITH IBRUTINIB, IDELALISIB OR VENEToclAX IN VITRO
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Background: CD19 is broadly expressed across B-cell malignancies, including chronic lymphocytic leukemia (CLL). MOR208 is an Fc-enhanced CD19 antibody mediating potent antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADOP) and direct cytotoxicity. Single agent MOR208 has shown promising activity in clinical studies.

Aims: We investigated the in vitro cytotoxicity of MOR208 when combined with the tyrosine kinase inhibitors (TKIs), ibrutinib and idelalisib, and the BCL-2 inhibitor, venetoclax.

Methods: The CLL cell line MEC-1 was treated with 0.3–10 µM ibrutinib, idelalisib or DMSO (control) for 7 days or 3–10 µM venetoclax or DMSO for 24 hours. Inhibition of proliferation, cytotoxicity and impact on CD19 expression were then assessed. ADCC assays with MOR208 incorporated a fixed number of primary human natural killer cells from healthy volunteers as effector cells. By contrast, the number of target cells was reduced according to antiprofiterative or cytotoxic effects of the TKIs or venetoclax. Dose-dependent ADCC activity of MOR208 was analyzed by flow cytometry. Cytotoxic effects were studied in at least three independent experiments.

Results: Ibrutinib and idelalisib induced only moderate direct cytotoxicity on ME1 target cells but had strong antiprofiterative effects. In contrast, venetoclax induced strong cytotoxicity on ME1 target cells within 24 hours. Both effects led to reduced tumor target cell numbers in the subsequent ADCC assays. CD19 expression was largely unaffected by all three drugs. The additional inhibition of idelalisib or venetoclax treated target cells resulted in enhanced maximum ADCC when compared with single agent MOR208. EC50 values remained unaltered in TKI or venetoclax treated conditions compared with the DMSO control. Calculations according to Chou-Talalay yielded combination indices below 1 for all three drugs, thus confirming synergistic activity.

Summary/Conclusions: The cytotoxic effect of MOR208 was synergistically enhanced when combined with ibrutinib, idelalisib or venetoclax in vitro. These promising data provide a strong rationale for combination of MOR208 with these agents in future clinical trials.

PB1769
LYMPHOCYTE EXHAUSTION AND THE NATURAL HISTORY OF CHRONIC LYMPHOCYTIC LEUKEMIA – FRIENDS OR FOES?
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Background: Chronic lymphocytic leukemia (CLL) is a disease characterized by the accumulation of morphologically mature monoclonal lymphocytes B with CD19+CD5+CD23+ phenotype in lymphoid tissue, peripheral blood and bone marrow. The course of CLL is chronic by default. Of note, however, is its heterogeneity. Programmed cell death protein 1 and its ligand 1 (PD-1, PD-L1) as well as CD200 and CD200 receptor (CD200R) are major inhibitory receptors of B and T cells.

Aims: We investigated the in vivo cytotoxicity of MOR208 when combined with the tyrosine kinase inhibitors (TKIs), ibrutinib and idelalisib, and the BCL-2 inhibitor, venetoclax.

Methods: The CLL cell line MEC-1 was treated with 0.3–10 µM ibrutinib, idelalisib or DMSO (control) for 7 days or 3–10 µM venetoclax or DMSO for 24 hours. Inhibition of proliferation, cytotoxicity and impact on CD19 expression were then assessed. ADCC assays with MOR208 incorporated a fixed number of primary human natural killer cells from healthy volunteers as effector cells. By contrast, the number of target cells was reduced according to antiprofiterative or cytotoxic effects of the TKIs or venetoclax. Dose-dependent ADCC activity of MOR208 was analyzed by flow cytometry. Cytotoxic effects were studied in at least three independent experiments.

Results: Ibrutinib and idelalisib induced only moderate direct cytotoxicity on ME1 target cells but had strong antiprofiterative effects. In contrast, venetoclax induced strong cytotoxicity on ME1 target cells within 24 hours. Both effects led to reduced tumor target cell numbers in the subsequent ADCC assays. CD19 expression was largely unaffected by all three drugs. The additional inhibition of idelalisib or venetoclax treated target cells resulted in enhanced maximum ADCC when compared with single agent MOR208. EC50 values remained unaltered in TKI or venetoclax treated conditions compared with the DMSO control. Calculations according to Chou-Talalay yielded combination indices below 1 for all three drugs, thus confirming synergistic activity.

Summary/Conclusions: The cytotoxic effect of MOR208 was synergistically enhanced when combined with ibrutinib, idelalisib or venetoclax in vitro. These promising data provide a strong rationale for combination of MOR208 with these agents in future clinical trials.

PB1770
HSP70 AND HSFl GO HAND IN HAND AND HAVE A ROLE IN THE SURVIVAL OF CHRONIC LYMPHOCYTIC LEUKEMIA NEOBLASTIC B CELLS
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Background: B-cell Chronic Lymphocytic Leukemia (CLL) is a neoplastic disorder characterized by the accumulation of clonal B cells in peripheral blood, bone marrow and lymphoid tissues. CLL is a clinically and biologically heterogeneous disease. As a consequence, novel biological and cytogenetic features have become increasingly important in predicting prognosis at the time of diagnosis and the research for molecules involved in apoptosis resistance and increased survival of neoplastic B cells is still ongoing.

Aims: We recently found that the Heat Shock Protein of 70kDa (HSP70) is overexpressed in Chronic Lymphocytic Leukemia (CLL) B cells. Considering the crucial survival role of HSP70 in cancer, we were aimed at characterizing this protein and its master regulator, the Heat Shock Factor 1 (HSFI), within the pathogenetic mechanisms leading to CLL.

Methods: HSP70 and HSFI expression levels were evaluated by Western blotting (WB) analysis in leukemic and normal B cells. HSP70 and HSFI protein levels were correlated to IGHV mutational status and ZAP70 protein expression in CLL patients. HSP70 and HSFI levels were also analyzed in neoplastic cells obtained from patients undergoing ibrutinib based regimen by WB analysis. Moreover, HSP70 and HSFI localization was analyzed by subcellular protein fractionation followed by WB analysis. The effects of HSP70 and HSFI inhibition by Zafirlukast and Fisetin were evaluated by Annexin V/Propidium iodide flow cytometry test and WB analysis of PARP cleavage.

Results: We demonstrated that HSP70 and HSFI are overexpressed in leukemic vs normal B cells and their expression levels correlate to poor prognosis in CLL. We also analyzed HSP70 and HSFI levels in patients following in vivo ibrutinib based regimen, observing a positive correlation between these two protein expression levels and moreover we observed that these two protein levels decreased after therapy. We found that at steady state both HSP70 and HSFI are localized in the nucleus of CLL B cells. HSP70 and HSFI inhibition was proved to be effective in inducing a dose-dependent in vitro apoptosis of CLL cells.

Summary/Conclusions: HSP70 and HSFI overexpression and correlation with poor prognosis in CLL patients underline their pivotal role in the regulation of leukemic B cell survival. HSP70 and HSFI both correlation and reduction in CLL patients following in vivo ibrutinib regimen let us hypothesize a role of these proteins in the progression of the disease. In normal B cells HSP70 and HSFI are both localized into the nucleus after stress conditions, however we found both HSP70 and HSFI localized into the nucleus of CLL B cells at steady state, suggesting a constitutive activation of these proteins in CLL. Although HSP70 has been extensively linked to cancer, little progresses have been made in bringing HSP70 inhibitors to the clinic, because of their potential off-target effects. For this reason we tried an alternative approach by targeting the HSP70 major regulator, HSFI. We observed that both inhibitors, Zafirlukast and Fisetin, lead to an in vitro dose dependent B cell apoptosis. These data demonstrate HSP70 and HSFI involvement in the pathogenesis of CLL and identify HSP70/HSFI axis as a target for new therapeutic strategies.
Background: Human concentrative nucleoside transporter 3 (hCNT3) belongs to a family of nucleoside transporters involved in fludarabine cellular uptake. It has been reported that overexpression of SLC28A3 gene encoding hCNT3 is a predictor of poor response to fludarabine-based chemotherapy. However, the mechanisms by which elevated expression of SLC28A3 mediates fludarabine resistance are still elusive.

Aims: The aim of the study was to examine possible influence of SLC28A3 gene overexpression on treatment response to fludarabine-cyclophosphamide therapy (FC) in patients with chronic lymphocytic leukemia.

Methods: We retrospectively analysed data from 54 CLL patients diagnosed and treated at Clinic for Hematology, Clinical Center of Serbia from 2003 to 2013. Blood samples were prospectively collected and analysed for biological and molecular features, as well as standard laboratory parameters. The expression of SLC28A3 gene was analyzed in peripheral blood mononuclear cells by RT-qPCR methodology, using TaqMan chemistry and ABI as endogenous control gene. Quantification of target gene expression was made by comparative ddCt method using HLA-BL cell line as the calibrator. All analyses were done prior to any treatment.

Results: Median age at diagnosis was 57 years (range 38-75). All patients were treated with fludarabine-based chemotherapy, 45 (83%) in the first treatment line. Overall response rate to the first line therapy was 81%, equally distributed on complete and partial responses (CR and PR), while the remainder included the same number of patients with stable disease (SD) and progressive disease (PD) (5, 9.6%). Most of the patients (42, 78%) relapsed during the follow up. At the time of the last follow up 14 (26%) patients were still alive, 35 (65%) were dead, and 5 (9%) were lost to follow up. Median overall survival was 76 months.

In the group of patients who received FC in the first treatment line (43/54), median expression of SLC28A3 mRNA in patients who experienced CR, PR, SD and PD was 0.036±0.030, 0.062±0.063, 0.030±0.025 and 0.157±0.257, respectively. The level of SLC28A3 expression was not associated with the IGHV mutational status. Patients who experienced PD to FC treatment overexpressed gene for hCNT3 compared to patients who achieved CR (p=0.013) and PR (p=0.05). We detected a significantly higher level of SLC28A3 expression in patients who experienced PD to FC treatment in comparison to patients who achieved CR (p=0.013) and PR (p=0.05).

Summary/Conclusions: Overexpression of SLC28A3 gene is a predictor of resistance to treatment with FC chemotherapy. Further studies are warranted to confirm these findings.

PB1772

THE SPECTRUM OF TP53, SF3B1, AND NOTCH1 MUTATIONS IN CHRONIC LYMPHOCYTIC LEUKAEMIA PATIENTS EXPOSED TO IONIZING RADIATION DUE TO THE CHORNOBYL NPP ACCIDENT

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Background: Generally, chronic lymphocytic leukemia (CLL) is considered to be a non-radiogenic form of leukemia. We previously found some clinical and biological features of CLL in group of clean-up workers of Chornobyl NPP accident indicated unfavorable disease course, such as high frequency of solid tumors and Richter transformation, mainly unmuted status of heavy chain variable region (IGHV) genes with increased usage of IGHV1-69 and IGHV3-21 (Abramenko et al., 2008). Analysis of genetic features of leukemic cells in IR-exposed CLL patients may provide an additional data on the possible causal relationship with IR.

Aims: The aim of the study was to analyze TP53, NOTCH1 and SF3B1 mutations in CLL patients, sufferers of Chornobyl NPP accident to clarify the possible pathogenetic relationship between IR and CLL development.

Methods: TP53, NOTCH1, and SF3B1 mutations were analyzed in 106 CLL patients who have been exposed to ionizing radiation (IR) due to Chornobyl NPP accident (83 clean-up workers, 16 inhabitants of radionuclide contaminated areas, and 7 evacuees) and in 130 IR non-exposed CLL patients as the control group. TP53 gene mutation analysis was performed for exons 3 to 10. NOTCH1 mutations and SF3B1 mutations were analyzed in the hotspot regions of these genes were the vast majority of CLL-specific lesions were reported: in c.711C>A (p.R237H), c.711C>T, c.711G>A of NOTCH1 gene, and in exons 14, 15 and 16 of SF3B1 gene, correspondingly.

Results: We found TP53 and SF3B1 mutations with similar incidence in both groups – in 11.3% and 10.0% of IR-exposed patients, and in 12.7% and 11.5% of IR non-exposed CLL patients, respectively. In contrast, NOTCH1 mutations were significantly more frequent in IR-exposed patients in comparison with the control group (6.7% vs 17.7%; p=0.012). Some other features were found among IR-exposed CLL patients also. Specifically, TP53 mutations were seen with equal frequency among mutated (11.1%) and unmuted (11.8%) IGHV cases in IR-exposed CLL patients, while the tendency to prevalence of TP53 mutations in unmuted compared with mutated IGHV cases was found in the control group (14.1% and 5.6%, correspondingly; p=0.178).

In IR-exposed group SF3B1 mutations were combined with mutations in TP53 almost in half of detected cases. In opposite, in the control group we observed reported earlier mutual exclusivity between SF3B1 and TP53 lesions (p=0.001 in comparison between observed groups). Among IR-exposed CLL patients we found two different cases with identical rare mutation of TP53 gene - c.665C>T substitution leading to change proline for leucine at codon 222 (Pro222Leu). This substitution is very likely to represent inherited TP53 mutation, which may influence CLL development under IR exposure.

Summary/Conclusions: In summary, our data suggest that TP53 abnormalities are involved in CLL development in sufferers of the Chornobyl NPP accident and also a possible interaction between inherited IR sensitivity caused by mutation in TP53, radiation and CLL development.

Background: Personalized Cancer Medicine is rapidly developing field that includes predictive medicine, preventive medicine and various personalized or individualized therapies, e.g. labeled "precision medicine". One particular challenge is that cancer is origin of each cancer is a clonal event evolving into tumor heterogeneity. We focus on Chronic Lymphocytic Leukemia (CLL), Multiple Myeloma and Follicular lymphoma (FL) that are currently considered incurable. Although current treatment regimens achieve sustained responses in a significant percentage of patients, CLL and MM cancer eventually relapse. Current challenges in using therapeutics against CLL and MM includes design of optimal treatment for individual patients based on characterization the tumor and its intratumor heterogeneity as observed by whole genome sequencing. Efficient therapies require a personalized approach that combines targeting lymphoma cells and the tumor microenvironment by restoring the patient’s own anti-tumor immunity. One solution to this challenge is the so-called "n-of-one" studies where protocols are organized with diagnostically based patient stratification to individualized treatment approaches (n=1).

Aims: To introduce individualized treatment for patients available therapies, we aim to established cell-based assays and drug sensitivity platform at NCM, University of Oslo and Oslo University Hospital. To establish a pipeline for direct drug sensitivity screening in CLL and MM (WP1-Path A). To Complement the results from WP1-Path A with Signaling pathway analysis (WP2-Path B) towards testing in xenografted mice and implementing therapy in n-of-one clinical trials. To offer patients with intractable CLL and MM individualized treatment with an effective combination of targeted therapies.

Methods: We culture CLL cells with combination of feeder cells that express CD40 ligand, CD27 ligand and CD38 ligand. Cells are cultured using C4-1 cell line (CD40 ligand, CD27 ligand and CD38 ligand). Cells are stimulated with anti-CD38 mAb (Becton Dickinson), anti-CD40 mAb (Becton Dickinson) and anti-CD27 mAb (Becton Dickinson). Cells are cultured in RPMI 1640 medium supplemented with 10% FCS. Cells are cultured under standard conditions for 72 hrs and 5 days) using cell proliferation, CellTox-Green assay for M2 cells has been performed. 7-AAD/BrdU cell proliferation and Caspase8/9 apoptosis assay was performed for flow cytometry (7-AAD/BrDU cell proliferation and Caspase8/9 apoptosis assay). ELISA measurements were performed for flow cytometry. CellTox Green assay for CLL cells (unstimulated and soluble CD40 ligand) has been performed. ELISA measurements were performed for flow cytometry. CellTox Green assay for M2 cells has been performed. Cell proliferation assay, CellTox Green, apoptosis and oxidative stress (glutathione release) are also performed. We also use established cell barcoding on CLL/MM for flow cytometry (7-AAD/BrDU cell proliferation and Caspase8/9 apoptosis assay).

Results: We culture CLL cells with combination of feeder cells that express CD40L, APRIL and BAFF for 24 hrs stimulation. We perform drug sensitivity screening with Prem Stimulated CLL cells in 384 well formats without feeder cells. We culture MM cells in 384 well plate for drug screening in response to TNFα Cells prestimulation in the presence of IL2. To support high-throughput drug sensitivity screening, we use cell-based assays such as CellTiter-Glo® Endpoint measurement using CellTiter-Glo assay has been performed for MM/CLL cells. Time course measurement using cell proliferation, CellTox-Green assay for CLL cells (unstimulated and soluble CD40 ligand) has been performed. Cell proliferation assay, CellTox-Green assay for M2 cells has been performed. Benzalkonium chloride (BzCl2) is used as Positive control. Endpoint measurement using CellTiter-Glo assay for CLL and MM cells was performed with cell density of 5000. Dose Response curve for 50 drugs has been generated for CLL patients (n=4) and MM (n=4) (Figure 1).

Figure 1.

Summary/Conclusions: We perform drug sensitivity screening to select potential drug candidates and pathway inhibitors through an approach where we directly assess patient samples. Selected drug candidates will first be validated by bioassays and the drug candidate to assess effects on intracellular mitogenic pathways (phosphoflow-based approach). We propose to use the drug sensitivity screening platform to identify and validate drug candidates for xenografting and "n-of-one" clinical trial studies.
Background: Chronic lymphocytic leukemia (CLL) is the most common form of adult leukemia in Western world with highly variable clinical outcome. Rituximab is a monoclonal chimeric anti-CD20 agent, that has demonstrated significant benefit for patients with different form of B cell lymphoproliferative disorders. Chemoinmunotherapy with rituximab, fludarabine and cyclophosphamide (R-FC) has shown to prolong progression free survival (PFS) and overall survival in CLL patients compared with chemotherapy alone. FCGR2A is polymorphic and has two alleles, FCGR2A-131H and FCGR2A-131R. This polymorphic variation is due to a single base substitution of nucleotide adenosine for guanine in position 49. FCGR2A-131H allele has a higher affinity for human IgG2, comparing to FCGR2A-R131. The gene for FCGR3A has also two polymorphic variant alleles: 158 valine (V158) and phenylalanine (F158) due to single base substitution of timidine to guanine at nucleotide position 559. FCGR3A-158V variant has higher affinity for Fc gamma receptor than 158F variant. These Fc gamma receptor polymorphisms may influence antibody-dependent cytotoxicity (ADCC), complement-dependent cytoxicity (CDC) and direct proapoptotic effect.

Aims: The aim of our study was to investigate a possible association of these two FCGR2A and FCGR3A variants with response to R-FC therapy in CLL patients.

Methods: We have analyzed these two polymorphisms in 90 patients with CLL treated with R-FC regimen. Median age of our patients was 62.36-78 and 63% were male. Number of patients with stage III/IV disease was 65(72%) and median WBC count at the start of treatment was 68.534-173x10(9). Percentage of previously treated patients was 51/90 (56.6%). Average numbers of R-FC cycles were 4.3 and median PFS was 35.1 months. Median time of observation after treatment was 3.6 years (range:6 months-8 years). Response was evaluated 2 months after therapy according to National Cancer Institute (NCI) criteria. Complete response (CR) was achieved in 24/90 (26.7%), partial response (PR) in 56/90 (62.2%) and no response in 10/90 (11.1%). DNA was isolated from peripheral blood mononuclear cells and genotyping was performed by using PCR/RFLP methods. The distribution of genotypes was compared by using a chi-squared test or Fisher's exact test.

Results: The distribution of genotypes in our study was: 32.5% H/H, 49.5% H/R and 18% R/R for FCGR2A and 43% V/V, 40% V/F and 17% F/F for FCGR3A. Rate of CR and PR were similar irrespective of the FCGR variants and our results did not demonstrate significantly different genotype distribution for FCGR2A (p=0.8001) or FCGR3A (p=0.1019) in CLL patients with complete, partial or no response to R-FC therapy (Table 1).

Table 1. Genotype distributions for FCGR2A & FCGR3A in patients with CLL.

<table>
<thead>
<tr>
<th>FCGR2A/FCGR3A</th>
<th>Complete Response n=24 (03.7%)</th>
<th>Partial Response n=56 (62.2%)</th>
<th>No Response n=10 (11.1%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCGR2A-131H/1</td>
<td>R2(6.0%) (162.7%)</td>
<td>16(09.0%) (293.9%)</td>
<td>4(3.5%) (536.2%)</td>
<td>0.8001</td>
</tr>
<tr>
<td>FCGR2A-131R/1</td>
<td>6(37.5%)</td>
<td>29(16.5%) (536.2%)</td>
<td>4(3.5%) (536.2%)</td>
<td></td>
</tr>
<tr>
<td>FCGR2A-131H/2</td>
<td>-</td>
<td>16(09.0%) (293.9%)</td>
<td>4(3.5%) (536.2%)</td>
<td></td>
</tr>
<tr>
<td>FCGR2A-131R/2</td>
<td>-</td>
<td>29(16.5%) (536.2%)</td>
<td>4(3.5%) (536.2%)</td>
<td></td>
</tr>
<tr>
<td>FCGR2A-131H/V</td>
<td>R2(6.0%) (162.7%)</td>
<td>16(09.0%) (293.9%)</td>
<td>4(3.5%) (536.2%)</td>
<td>0.1019</td>
</tr>
<tr>
<td>FCGR2A-131R/V</td>
<td>-</td>
<td>16(09.0%) (293.9%)</td>
<td>4(3.5%) (536.2%)</td>
<td></td>
</tr>
<tr>
<td>FCGR3A-158V/V</td>
<td>R2(6.0%) (162.7%)</td>
<td>16(09.0%) (293.9%)</td>
<td>4(3.5%) (536.2%)</td>
<td></td>
</tr>
<tr>
<td>FCGR3A-158F/V</td>
<td>-</td>
<td>16(09.0%) (293.9%)</td>
<td>4(3.5%) (536.2%)</td>
<td></td>
</tr>
</tbody>
</table>

Summary/Conclusions: Our results are similar with previously reported results in other studies in CLL patients, but in contrast with the results for follicular lymphoma (FL), which showed that high-affinity FCGR2A-158V/V variant was associated with the highest response rates in FL patients treated with rituximab. These findings could be explained with the different mechanism of action of rituximab in CLL compared to lymphoma patients or could be due to the variations in selected patients' population.
Chronic lymphocytic leukemia and related disorders

PB1776

LAMBDA LIGHT CHAIN RESTRICTION – USEFUL FOR HAIRY CELL LEUKAEMIA PROGNOSIS?

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Background: Hairy cell leukemia (HCL) patients have near-normal life expectancies since the introduction of purine nucleoside analogues. However, HCL remains a chronic, often relapsing disease in which maximizing treatment-free survival (TFS) is the main goal.

Aims: Prognostication is not standardized in HCL, emphasizing the relevance of the characterization of HCL populations.

Methods: We retrospectively analysed 40 patients (90% men), diagnosed between 1997 and 2016, with a median follow-up of 6 years.

Results: At presentation, the median age was 58 years and 69% of patients were symptomatic - fatigue (53%), B symptoms (50%), bleeding (14%), abdominal discomfort (6%) and severe infection (22%). The commonest cytopenia was thrombocytopenia (70%), with median platelet count being 66x10^9/L. Monocyte counts below 0.1x10^9/L were observed in 61% of patients. Splenomegaly was observed in 83% of the patients and 21% had abdominal lymphadenopathies. The majority of the patients (88%) was treated with cladribine in first line, achieving an overall response (OR) rate of 100% and a complete response (CR) rate of 38%, of which 67% were classified as minimal residual disease (MRD)-negative CR. Retreatment was required in 33% of the patients, of which the majority received cladribine. The median time-to-next-treatment (TNT) from first to second line treatment was 91%, 50% achieving CR, of which 33% were classified as MRD-negative CR. Only 5% of the patients required further treatment lines. Even the presence of scarce hairy cells in the bone marrow precluded classification of response as CR. This might have contributed to the low CR levels observed in our patients. As post-treatment bone marrow biopsies were available in only 24 patients, response analysis was restricted to these patients. All of these 24 patients had bone marrow fibrosis at diagnosis, which reverted when and in whom first CR was obtained. Median overall survival (OS) was not reached and, at 10 years, the OS was 90%. Four deaths occurred, all unrelated to HCL. Regarding prognostication, a trend to a longer TFS, albeit not statistically significant, was observed in patients achieving CR (namely MRD negative) and without thrombocytopenia at presentation. Excitingly, the 61% of patients with lambda (λ) light-chain restriction (LCR) displayed a significantly higher TFS than the 39% with kappa (κ) LCR (p=0.04, Wilcoxon-Gehan test). To the best of our knowledge, there are no published reports on prognostic value of LCR in HCL (Figure 1).

Summary/Conclusions: If multicentre studies corroborate our findings, LCR may be of use in the prognostication/risk stratification of HCL. Similarly with multiple myeloma and other hematological malignancies, lambda (λ) LCR appears to correlate with worse prognosis, leading to a shorter TFS.

PB1777

CLINICAL EFFICACY AND LONG-TERM OUTCOMES OF SPLENECTOMY IN CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background: Chronic lymphocytic leukemia (CLL) is often accompanied by splenomegaly, which may increase to such a degree that causes abdominal discomfort, regional portal hypertension, and becomes a place of malignant cells concentration. In 2.3-4.3% of cases CLL may be complicated by autoimmune cytopenias (autoimmune hemolytic anemia (AIHA), immune thrombocytopenia (ITP), Evans-Fisher syndrome). Accordingly, the effectiveness of steroid and chemotherapy in such cases may be impaired, raising the question of splenectomy advisability.

Aims: To analyze splenectomy effectiveness in patients with CLL.

Methods: Splenectomy was performed in 41 patients with CLL, 12 of which were patients with CLL and ITP, 9 with CLL and warm type AIHA, 5 patients with CLL and Evans-Fisher syndrome, along with 15 CLL patients without immune disorders. Among the patients there were 26 males and 15 females. Indications to splenectomy were following: massive splenomegaly with abdominal discomfort, immune cytopenia and regional portal hypertension. In one female patient the surgical intervention was performed urgently due to spontaneus splenic rupture and acute intra-abdominal bleeding.

Results: Splenectomy was effective in 37 patients (90.2%): abdominal discomfort disappeared, hemolysis stopped and hemoglobin levels normalized or increased, platelets numbers normalized or increased. Splenectomy was ineffective in 3 patients with CLL associated with ITP: amid elimination of abdominal discomfort the platelets number did not increase significantly (2 patients), while in 1 patient despite increase in platelets number leukemia progression was observed. One (2.4%) patient with CLL and AIHA died on 3rd day after surgery because of acute adrenal insufficiency. The analysis of late effects of splenectomy in patients with CLL showed that average life expectancy after the surgery comprised 111.6 months within observation period between 11 and 277 months. In patients with CLL and immune cytopenias the average life expectancy after surgery was shorter and equal to 60.7 months within the observation period between 2 and 361 months.

Summary/Conclusions: Splenectomy remains an effective method of treatment of patients with CLL associated by severe splenomegaly and immune cytopenia. Long-term results of splenectomy in patients with CLL without cytopenia are better than in patients with CLL and cytopenias. Aggressive hemolysis, large spleen covered in perisplenitis adhesions, amid portal hypertension and thrombocytopenia are considered to be special surgical risk factors in this patients.
22nd Congress of the European Hematology Association
influenza vaccination (RR 10.47; 95%CI 2.54-43.07; p=0.003) were associated
with increase risk of developing MBL. After adjusted for age, only history of
influenza vaccination and family history of LPD were an independent risk factor
for developing MBL with age adjusted RR of 9.75 (95%CI 2.3-40.5; p=0.002)
and 92 (95%CI 56.3-149.5; p<0.001), respectively.
Summary/Conclusions: MBL prevalence in Thai population is much lower
than previously reported. It more frequent in elderlies and associated with
family history of LPD and influenza vaccination. Although uncommon, the
presence of high-count MBL warrants further investigations to define the biological and clinical significance in term of LPD transformation and long-term
survival.
PB1779

SPONTANEOUS CLINICAL REGRESSION IN CHRONIC LYMPHOCYTIC
LEUKEMIA: CLINICAL AND BIOLOGIC FEATURES OF 9 CASES FROM
THE ERIC REGISTRY
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Background: Spontaneous clinical regression in chronic lymphocytic leukemia
(CLL) is rare (1% per year). We previously reported on the clinico-biologic features of 9 Binet stage A CLL patients from our Center in Rome who experienced
a persistent spontaneous clinical regression of the disease at a median time
of 11 years from diagnosis, maintained after 5 more years of follow-up. The
lymphocyte count at CLL regression was 3.16 x 109/L (1.3-4.9), with a persistent
small CLL clone (CD19+/CD5+/CD23/light chain restricted: 44%, range 5-60%).
Biologic features included negative CD38, mutated IGHV, often with VH3-30
and Vk4-1 usage, and a distinctive gene expression profile.
Aims: To conduct a retrospective collection of clinical data and basic biologic
information on CLL spontaneous regressions and to make them accessible for
future research.
Methods: A registry of spontaneous CLL regressions (absence of lymphadenopathy, splenomegaly or constitutional symptoms, peripheral blood (PB)
lymphocytes <4 x 109/L, in the absence of any previous treatment) was
launched within the ERIC consortium.
Results: So far, 9 CLL patients showing a spontaneous regression have been
reported and 8 have been formally registered, 7 from Italy and 2 from Sweden.
Six were males and 3 females, with a median age of 57 years at diagnosis
(range 51-82), stage Binet/Rai A/0 in 6, A/I in 2 and B/II in 1. The median lymphocyte count at diagnosis was 14.1 x 109/L (5.3-51.9). Biologic features included: mutated IGHV in 8/8 with VH3-30 (2), VH3-21, VH3-15, VH3-23, VH4-31,
VH4-34, VH4-59; CD38 <30% in 6/6; ZAP70 <20% in 4/6; FISH (7 cases):
del13q in 4, negative in 3, +12 in 1 case. No patient had undergone treatment,
except for one diagnosed in 2009 who received FCR for disease progression
in 2013 (lymphocytes 107 x 109/L), obtained a PR and 18 months later developed a Richter’s syndrome - a diffuse large B-cell lymphoma clonally unrelated
to CLL - with the concomitant disappearance of the CLL clone from the PB and
bone marrow, that has lasted up to January 2017 (lymphocytes 3.5 x 109/L,
CLL 0.035 x 109/L). An additional case diagnosed in 2013 (stage A/I, lymphocytes 37.2 x 109/L) reached the highest lymphocyte count 19 months later
(91.2 x 109/L) and subsequently started a spontaneous reduction in lymphocytosis down to 39.6 x 109/L in 2015 and to 8.9 x 109/L in January 2017 in
stage A/0, indicative of a partial but ongoing CLL regression. Excluding the
latter cases, in the other 7, all in stage A/0, the highest lymphocyte count was
16.0 x 109/L (8.9-76.0), the lowest at the last follow-up was 2.8 x 109/L (1.84.4), with 0.66 x 109/L CLL cells (0.085-3.0) in the 4 evaluable cases. The
median time from diagnosis to clinical regression was 4 years (range 2-17)
and this has been maintained for 2 further years (range 0.5-7). One of these
cases (mutated VH3-21, +12) seems the most dramatic: in 2008 at diagnosis,
the lymphocytes were 51.9 x 109/L, in 2009 a peak at 76.0 x 109/L was recorded; in 2011, when the CLL regression started, the patient underwent several
mild viral upper respiratory infections; the CLL complete regression (1.8 x 109/L)
persists up to the last follow-up. In 5/9 cases one event - mild viral infections,
a cerebral hemorrhage, a stroke, a pelvis fracture and a Richter’s syndrome occurred before the spontaneous regression, but no relevant drug intake was
recorded.
Summary/Conclusions: Clinicians should be aware that spontaneous regression is a possibility, albeit infrequent, in the natural history of CLL. The collection
and study of such cases within the ERIC registry may shed light on mechanisms
leading to spontaneous regression and critical pathways in immunosurveillance
in CLL.

714 | haematologica | 2017; 102(s2)

PB1780

CLINICAL AND LABORATORY CHARACTERIZATION OF PLATELET
DYSFUNCTION DURING IBRUTINIB TREATMENT IN PATIENTS WITH
CHRONIC LYMPHOCYTIC LEUKEMIA. MONOCENTRIC EXPERIENCE
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Background: Ibrutinib (IBR) is a potent and irreversible inhibitor of Bruton’s
tyrosine kinase (Btk) approved by FDA for the treatment of patients (pts) affected by chronic lymphocytic leukemia (CLL) with del 17p or TP53 mutation or for
pts with relapsed/refractory (R/R) CLL. IBR is associated with bleeding events
usually mild (Common Toxicity Criteria (CTC) grades 1-2), rarely severe (grade
3-4). A defect of platelet function, namely an inhibition of Btk-mediated signaling
by platelet glycoproteins (GP) GPVI and GPIb, has been hypothesized to cause
these bleedings. IBR associated bleedings and platelet dysfunction may be
relevant in CLL pts who are usually elderly and with comorbidities requiring
antithombotic therapies.
Aims: To investigate and characterize the effect of IBR on platelet function in
pts with CLL.
Methods: We enrolled from May 2014 to December 2016 twenty pts with
CLL treated with orally administered 420 mg daily of IBR; 18 R/R CLL pts
received IBR in monotherapy and 2 pts with previously untreated CLL
received IBR in association with anti-CD20 MoAb. Median age was 68 years
(57-84); 13 pts had unmutated IgVH and 2 had 17p deletion. The median
number of prior therapies in R/R CLL pts was 3 (2-7). Five pts discontinued
IBR therapy: 2 for Richter’s transformation, 1 for progressive CLL, 1 underwent allogeneic HSCT, 1 for heart disease. The platelet function was studied
before and during IBR by light transmission aggregometry (LTA) using
platelet-rich plasma and the following agonists: ADP 2-4 uM, PAR1-AP 25
uM, Collagen 10-3.3-2 ug /mL, arachidonic acid 1 mM, ristocetin 0.6-1.2
mg/mL. Also measurements of von Willebrand factor antigen (vWF:Ag) and
ristocetin cofactor activities (RiCo) by chemiluminescent immunoassay were
performed. All pts had measurements of the platelet function at the baseline
and after 1, 3, 6 months initiation of IBR and then every 3 months up to 24
months. Median observation period was 9 months. No patient received concomitant antiplatelet or anticoagulation therapy.
Results: Nineteen pts achieved a partial response and an increase of hemoglobin and platelet count. We recorded CTC grade 1 or 2 bleedings (bruising,
petechiae, conjunctival and retinal hemorrhage, rectal bleeding) in 15 pts; no
patient needed IBR interruption or dose reduction. All pts displayed severe
impairment of collagen induced aggregation upon IBR. Reduction of maximal
aggregation (35.6+/- 32% vs 70.6+/- 21% baseline) and prolongation of the
lag phase (261+/- 54 sec vs 72+/- 26.8 sec baseline) by 2 ug/mL collagen was
measured in all pts during IBR. In 10 pts a significant improvement of the aggregation by 2 uM ADP (71+/- 31.8% vs basal 48.6+/- 31%) and 4 uM ADP (84+/11% vs basal 64+/- 25%) was found during IBR. The aggregation by 25 uM
PAR1-AP, 1.2 mg/ml ristocetin and 1 mM arachidonic acid was unchanged
before and under IBR. Finally, in 9 pts the vWF:Ag and RiCo were high at the
onset of the disease (163+/- 59.8% and 181.6+/- 82.5%) and reduced up to
normal values under IBR (118+/- 71% and 145+/- 65%).
Summary/Conclusions: Our study showed minor bleedings in pts treated
with IBR. A severe impairment of collagen-induced aggregation was caused
by IBR, which was counteracted by amelioration of ADP-induced aggregation,
that could explain, at least partially, the mild clinical phenotype in treated pts.
The assessment of platelet function in IBR treated CLL pts could help to predict
and monitor the bleeding risk, and to guide pts through invasive procedures.
In addition, pts under anticoagulant or antiplatelet treatment might need be
carefully monitored by clinical and laboratory evaluation.
PB1781

HAIRY CELL LEUKEMIA :A SUMMARY OF CLINICAL DATA ON 202
PATIENTS AND THE RESULTS OF THERAPY WITH CLADRIBINE IN
ISRAEL
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Background: Hairy cell leukemia (HCL) accounts for approximately 2% of all leukemias and is associated with pancytopenia, splenomegaly, and recurrent infections. Therapy with the purine analogues cladribine (2CdA) or pentostatin (2’deoxycoformycin), has been most effective and both agents have achieved equivalent results in HCL. In this regard cladribine given as a single course, achieves a high response rate. Several alternative dosing schedules have been reported (Table 1). Cladribine was administered either as a “fixed daily dose” or “weight based dose” for 5 or 7 days. Seeing that excellent results are obtained using 2CdA in all schedules used, it now seems very important to focus on reducing therapy induced toxicity, related mostly to development of neutropenia, immunosuppression and severe infections.

AIM: In this prospective study, we have summarized the Israeli experience with HCL over the past 30 years, and analyzed demographic data, relevant laboratory and clinical parameters with special emphasis on outcome after first line treatment with cladribine.

Methods: We collected retrospective data on patients with HCL from 12 medical centers in Israel, followed and treated during 1985-2015. The study was approved by local institutional IRBs of each medical center.

Results: Data from the medical records of 202 patients with HCL was summarized. Mean follow up was 7.5 years (0.1–40); with a 5 and 10 years’ overall survival of 96% and 90.62% respectively. The median age at diagnosis was 53 years, and most (81.77%), were males. In terms of ethnicity: 88.3% of patients were Jews with (52.2% Ashkenazi and 36.1% Sephardic Jews) while 11.7% were Arab, Druz or others. First line therapy with cladribine was given to 159 patients (80.71%); other therapies 9.14%, while 1.1% did not receive any treatment. The median time from HCL diagnosis to treatment with 2CdA was 5.9 years. IV therapy was given to 62% of patients and 38% received it SC. Complete remission rates, progression-free survival and overall survival were not significantly different between the two schedules. In univariate analysis: Sex, ethnicity, dose, patient weight, and treatment duration (5-7 days) had no impact on outcome, but patients older >65 years had a shorter survival. Infectious complications requiring hospitalization was reported in 50.3% of all treated patients (54%, post IV and 47% post SC delivery; p=0.04). Median days of hospitalization were 8 for both groups (0-45) (p=0.05), and the length of NADIR was 18 and 20 days for IV and SC delivery respectively (p=0.33).

Summary/Conclusions: This is the first comprehensive summary of the national experience involving a large cohort of HCL patients with long follow up. These results serve as validation of previous reports relating to HCL and confirm that the excellent outcome achieved after a single course of treatment with 2CdA is independent of schedule and method of drug delivery. In addition, patient ethnicity was insignificant.

PB1782

CHRONIC LYMPHOCYTIC LEUKEMIA: CHANGES IN CLINICAL STAGE DISCRIMINATE PATIENTS WITH DIFFERENT OUTCOME WITHIN THE IWCLL PARTIAL RESPONSE CATEGORY

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Background: Over the last decades, progress in chronic lymphocytic leukemia (CLL) treatment has resulted in an impressive increase in overall survival (OS). In CLL, as in other tumors, response to therapy overcomes negative prognostic factors and is the most important predictor of survival. Clinical stages reflect tumor load and correlate with OS both at diagnosis and over the course of the disease (Rai et al, Blood 1975).

Aims: To determine whether changes in clinical stage discriminate patients with different outcome within IWCLL response categories, particularly the heterogenous subcategory partial response (PR) group (Bennell et al. Blood 2008).

Methods: Two-hundred ninety-nine patients with CLL were retrospectively evaluated. Median follow up was 91 months (range, 2-390). CLL diagnosis was based on IWCLL criteria. Endpoints were time to next treatment (TTT) and OS. TTT and OS curves were estimated by the Kaplan-Meier method and differences were determined by log-rank test. Results were analyzed according to IWCLL recommendations and by changes in clinical stage. A landmark analysis was performed in ninety-two patients in whom a PR was achieved at any time during the course of the disease.

Results: From the whole series of 229 patients, those who achieved a better IWCLL degree of response after first line therapy had a better OS than those with an inferior response (p<0.001). With a median follow up of 91 months (range, 2-390), the median survival in patients who achieved complete remission (CR) was 214 (95% CI: 123-305) vs 134 (95% CI: 79-189) and 91 (95% CI: 36-146) months, respectively in those who achieved PR and failed to therapy, respectively (Figure 1A). Among patients in PR (n=66), after a median follow-up of 42.5 months (range 1-201), those patients with stage A disease at the time of response evaluation (PR Binet A) had significantly better outcome than those whose stage was Binet B/C (median survival 63 vs 43 months; p=0.047). Interestingly, when the analysis was restricted to response assessment after first line therapy (n=229), patients who achieved PR Binet A did not have significant differences in OS compared to those patients who were in CR (median survival were 164 and 214 months respectively; p<0.001); on the contrary, patients in PR Binet B/C had a similar outcome than those who did not respond to treatment (median survival 81 and 91 months respectively; Figure 1B). Similar results were observed in the outcome of patients with PR subclassified according to Rai clinical stage.

Figure 1.

Summary/Conclusions: Changes in clinical stage provide reliable information on disease evolution and is associated with therapy in patients with CLL, particularly those in the IWCLL PR category. This study supports the use of clinical stages as a complementary and simple tool to assess response in patients with CLL, both at the end and over the course of treatment.

PB1783

INCIDENCE OF THYROID GLAND DISORDERS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Frequency of autoimmune complications like immune anemia or immune thymocytopenia has increased in patients with chronic lymphocytic leukemia (CLL). However, there is no data in the literature investigating the relation of the other autoimmune disorders including thyroid gland diseases with CLL.

Aims: We aimed to investigate the presence, features and frequencies of thyroid disorders in patients with CLL.

Methods: Thyroid function tests, thyroid autoantibodies (antithyroglobulin antibody [anti-TG], antithyroid peroxidase antibody [anti-TPO]), thyroid ultrasonographies (USG) and scintographies of CLL patients were performed. Demographic data, Rai-stages, and establisment of thyroid disorders were recorded.

Results: One hundred CLL patients were included into the study (65 male, mean age was 62±10.4). Free T3 (FT3) was within normal limits in 96 cases (96%), was low in 2 cases (2%), was high in 2 cases (2%); free T4 (FT4) was normal within normal limits in 89 cases (89%), was normal in 7 cases (7%), was high in 4 cases (4%); TSH was within normal limits in 90 cases (90%), was low in 7 cases (7%), was high in 3 cases (3%), Anti-TPO and anti-TG were positive in 10 cases (11.8%) and in 18 cases (21.2%), respectively. While USG was normal in 36 cases, multinodular goiter (MNG) in 21, chronic thyroiditis in 20, MNG associated with thyroiditis in 10, uniodular goiter (UNG) in 8, UNG associated with thyroiditis in 4, and diffuse goiter in 1 case were determined by USG. Toxic adenoma in 3 cases, toxic MNG in 2 cases, and thyroiditis in 1 case were determined in 6 patients in whom thyroid scintigraphy was performed for hyperthyroidism. After evaluation of all the tests; while no thyroid disease was determined in 33 of the cases (33%), MNG in 25 (25%), thyroiditis accord- ing to the results of USG in 12 (12%), UNG in 11 (11%), Hashimoto thyroiditis in 9 (9%), toxic MNG in 3 (3%), subclinical hyperthyroidism in 3 (3%) cases, subclinical hypothyroidism in 1 case (1%), lymphocytic thyroiditis in 1 case (1%), toxic UNG in 1 case (1%), and euthyroid sicknes syndrome in 1 case (1%) were determined. The patients were divided into 2 groups according to their Rai-stages and ages. Accordingly; Rai-stage 0 - I - II (n=80) and Rai-stage III - IV (n=20), <65 years (n=56) and ≥65 years (n=44). Anti-TPO positivity was similar in 2 Rai-stages groups and in both sexes (p=0.507, p=0.223, respectively). However, anti-TPO positivity was statistically different between age groups; anti-TPO was positive in 3 patients in <65 years old age group, and was positive in 7 patients in ≥65 years old age group (p=0.049). Anti-TG was positive in 7 patients in <65 years old age group,
PB1785

CLINICAL-BIOLOGICAL CHARACTERISTICS, TREATMENT OUTCOME AND SURVIVAL OF SMALL LYMPHOCYTIC LYMPHOMA PATIENTS: A REAL-LIFE EXPERIENCE

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Methods: Cases with lymph node (LN) involvement by SLL in which proliferation centers (PCs) were not observed and pts in whom lymphadenopathy was <1.5 cm showing a better prognosis

Aims: To: a) record clinical, biological features and treatment strategy in a series of SLL pts diagnosed in our centers b) correlate clinicopathological characteristics and treatment with response and survival c) detect possible differences in terms of response and survival between SLL pts according to LN characteristics (size of LN and presence of PCs)

Methods: Pts diagnosed with SLL from 2007 up to now fulfilling the diagnostic criteria of SLL in 2008 WHO classification were included. Clinical and biological data were recorded at diagnosis as well as treatment related variables, such as type of treatment, response and patient survival. Moreover, LN features such as the size, and the presence of PCs were also studied. PCs were not observed and pts in whom lymphadenopathy was <1.5 cm showing a better prognosis

Results: 47 pts were analysed. Pts’ median age was 69yo (range, 40-87) with no gender predominance (24male/23female). According to Binet staging system 12, 19 and 9 were classified as A, B and C stage respectively while according to Rai staging system 19 and 9 were classified as A, B and C stage respectively. LN biopsies were performed in 37 out of 47 pts. All pts underwent bone marrow (BM) biopsy with a median BM infiltration of 45% (0-97%). PCs were identified in 19 out of 24 cases with lymphoid blasts showing a better prognosis

Conclusions: The study was proposed to 91 consecutive patients, independently of age, level of education and internet accessibility, and 72.5% of patients agreed to participate to the study. The main reason of refusal was lack of time (52%), lack of interest to participate in the project (22%), lack of knowledge (12%) and health-related reasons (6%). In cases with no access to internet, but with interest to participate in the project, the questionnaires were administrated through tablet, before the scheduled visit, by a dedicated nurse. Patient compliance: a global response of 85.2% was observed; 48 patients responded at least once and 23 at all scheduled time points. In each case, all questionnaires were fully completed. At this timepoint we cannot yet evaluate system effectiveness as the study is still ongoing. However, ad interim data (Table 1) suggest that patients who interrupt questionnaires fulfilling are those with younger age, more intense working activity and experiencing no changes in disease status (e.g. untreated cases or those in remission). In particular, patients who were under treatment during the questionnaires administration period, showed a higher adherence compared to those in follow up, both previously treated or not (80% versus 26%, p<0.05).

Table 1.

PB1785

HEMINSIGHT TO ASSESS PATIENT REPORTED OUTCOMES OF PATIENTS AFFECTED BY CHRONIC LYMPHOCYTIC LEUKEMIA IN DAILY CLINICAL PRACTICE

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Aims: HemInsight was implemented at our Centre to collect PROs from CLL patients in daily practice.

Methods: HemInsight incorporated the EORTC QLQ-C30, EORTC QLQ-CLL 16, SF-36, and the 8-item Morisky Medication Adherence Scale (MMAS-8) questionnaires to collect PROs and their changes during various stages of CLL (diagnosis - progression - treatment). PRO assessments were scheduled for the patients who had in fact chronic lymphocytic leukemia. HemInsight, a project conceived in 2010 for myeloproliferative neoplasms in haematological centres in Denmark, enables patients to periodically submit PROs online to be combined to the medical records.

Results: At the time of the present report, 74 patients with a CLL diagnosis have been enrolled, 15 of whom were newly diagnosed. Fourteen patients underwent cytoreductive therapy and 2 are under treatment with novel oral drugs. System attrition: the study was proposed to 91 consecutive patients, independently of age, level of education and internet accessibility, and 72.5% of patients agreed to participate to the study. The main reason of refusal was lack of time (52%), lack of interest to participate in the project, the questionnaires were administrated through tablet, before the scheduled visit, by a dedicated nurse. Patient compliance: a global response of 85.2% was observed; 48 patients responded at least once and 23 at all scheduled time points. In each case, all questionnaires were fully completed. At this timepoint we cannot yet evaluate system effectiveness as the study is still ongoing. However, ad interim data (Table 1) suggest that patients who interrupt questionnaires fulfilling are those with younger age, more intense working activity and experiencing no changes in disease status (e.g. untreated cases or those in remission). In particular, patients who were under treatment during the questionnaires administration period, showed a higher adherence compared to those in follow up, both previously treated or not (80% versus 26%, p<0.05).

Summary/Conclusions: In conclusion, HemInsight is a useful tool for QoL evaluation in CLL patients. Provisional data suggest a higher compliance of those patients who feel that they need a closer contact with the clinician, both for individual disposition or disease status.
PB1786

HEALTHCARE COST OF MEDICARE PATIENTS WITH PREVIOUSLY TREATED CHRONIC LYMPHOCYTIC LEUKEMIA


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Background: Chronic lymphocytic leukemia (CLL) is the most prevalent form of leukemia in adults in the industrialized countries, accounting for 20% to 30% of all leukemia cases. CLL affects mainly elderly patients, with a median age at the time of diagnosis reported to be 71 years. Although CLL is not curable, disease symptoms and progression may generally be controlled with adequate pharmacologic treatments. Bendamustine-based regimens have long time been used in the management of CLL patients but few studies have analyzed the comorbidity- and/or adverse event (CAE)-related healthcare costs in elderly patients receiving these regimens in a real-world setting.

Aims: To describe all-cause and CAE-related healthcare costs of elderly patients with CLL treated with a bendamustine-based regimen in second or later lines of therapy or in the treatment of relapse. Healthcare costs were adjusted for inflation (2016 USD) and reported per-patient-per-month (PPPM).

Methods: A retrospective cross-sectional cohort study design was used. Adult patients who received a bendamustine-based regimen in second or later lines of therapy on or after January 2010 were identified from the Medicare Limited Data Set (LDS) 5% Standard Analytic Files (data availability: 1999–2014). The index date was defined as the initiation date for the first of the studied bendamustine-based regimens. Selected patients were required to be continuously enrolled in their Medicare plan for ≥6 months before and ≥3 months after the index date – unless the patient died during the first 3 months after the index date. Patient cohorts were determined based on the treatment initiated on the index date (index treatment); the two most prevalent bendamustine-based regimens were analyzed, i.e., (1) bendamustine and rituximab in combination (BR cohort) and (2) bendamustine monotherapy (bendamustine cohort). Healthcare costs, including inpatient, outpatient, and CAE-related costs, were calculated. The CAE-related treatment was described for each cohort. For each medical cost component, all-cause and CAE-related costs were summarized. Healthcare costs were adjusted for inflation (2016 USD) and reported per-patient-per-month (PPPM).

Results: A total of 275 patients were included in the BR cohort and a total of 100 patients in the bendamustine cohort. Most patients (61.8% in the BR cohort and 65.0% in the bendamustine cohort) were male and the mean age was approximately 75 years old. During the 6 months prior to the index date, patients in the BR and bendamustine cohorts were similar in terms of comorbidity profile; mean Charlson comorbidity index was 3.53 in the BR cohort versus 3.63 in the bendamustine cohort (p=0.581). During treatment in total all-cause healthcare costs were $14,520 PPPM for the BR cohort and $13,125 PPPM for the bendamustine cohort – outpatient costs (mainly driven by CLL-drug costs) represented the largest cost component. CAE costs accounted for a relatively large portion of the total all-cause healthcare costs; 58.3% for the BR cohort and 66.9% for the bendamustine cohort.

Summary/Conclusions: In this population of elderly patients previously treated for CLL, healthcare costs incurred during relapsed treatment with bendamustine-based regimens were high and a large portion of the costs were driven by comorbidity and/or adverse event-related costs. Results also suggest that the addition of rituximab to bendamustine does not appear to be a major cost factor.

PB1787

THE ROLE OF MAINTENANCE THERAPY IN THE TREATMENT OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: The inclusion in the treatment program of new drugs (including monoclonal antibodies and targeted therapies) allowed the majority of patients with chronic lymphocytic leukemia (CLL) to achieve disease remission (complete or partial) after combined therapy. So, at now, the urgent task is longer in the patients with MT

Aims: To describe all-cause and CAE-related healthcare costs of elderly patients with CLL treated with a bendamustine-based regimen in second or later lines of therapy or in the treatment of relapse. Healthcare costs were adjusted for inflation (2016 USD) and reported per-patient-per-month (PPPM).

Methods: A retrospective cross-sectional cohort study design was used. Adult patients who received a bendamustine-based regimen in second or later lines of therapy on or after January 2010 were identified from the Medicare Limited Data Set (LDS) 5% Standard Analytic Files (data availability: 1999–2014). The index date was defined as the initiation date for the first of the studied bendamustine-based regimens. Selected patients were required to be continuously enrolled in their Medicare plan for ≥6 months before and ≥3 months after the index date – unless the patient died during the first 3 months after the index date. Patient cohorts were determined based on the treatment initiated on the index date (index treatment); the two most prevalent bendamustine-based regimens were analyzed, i.e., (1) bendamustine and rituximab in combination (BR cohort) and (2) bendamustine monotherapy (bendamustine cohort). Healthcare costs, including inpatient, emergency room, outpatient and CAE-related costs, were calculated. The CAE-related treatment was described for each cohort. For each medical cost component, all-cause and CAE-related costs were summarized. Healthcare costs were adjusted for inflation (2016 USD) and reported per-patient-per-month (PPPM).

Results: A total of 275 patients were included in the BR cohort and a total of 100 patients in the bendamustine cohort. Most patients (61.8% in the BR cohort and 65.0% in the bendamustine cohort) were male and the mean age was approximately 75 years old. During the 6 months prior to the index date, patients in the BR and bendamustine cohorts were similar in terms of comorbidity profile; mean Charlson comorbidity index was 3.53 in the BR cohort versus 3.63 in the bendamustine cohort (p=0.581). During treatment in total all-cause healthcare costs were $14,520 PPPM for the BR cohort and $13,125 PPPM for the bendamustine cohort – outpatient costs (mainly driven by CLL-drug costs) represented the largest cost component. CAE costs accounted for a relatively large portion of the total all-cause healthcare costs; 58.3% for the BR cohort and 66.9% for the bendamustine cohort.

Summary/Conclusions: In this population of elderly patients previously treated for CLL, healthcare costs incurred during relapsed treatment with bendamustine-based regimens were high and a large portion of the costs were driven by comorbidity and/or adverse event-related costs. Results also suggest that the addition of rituximab to bendamustine does not appear to be a major cost factor.

PB1788

MULTISITE PERFORMANCE EVALUATION STUDY OF THE BD ONEOFLOW LYMPHOCYTE SCREENER 2 SYSTEM


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Background: The BD OneFlow solution for diagnostic screening of chronic lymphoproliferative disorders (CLPDs) includes a standardized flow cytometry approach based on the EuroFlow (EF) Consortium liquid reagent system. The BD OneFlow solution enables reproducible identification and discrimination of normal from aberrant mature cell populations by combining standardized assays, set reagents, and protocols. The BD OneFlow LST (Lymphoid Screening Tube) is intended for flow-cytometric immunophenotyping of normal and aberrant mature lymphocyte populations of B, T, and NK lineages in specimens (peripheral blood, bone marrow, and lymph node) from patients with hematological disorders. BD OneFlow LST acquisition and analysis template version 1.0 was revised to version 2.0 to include determination of lymphocytes as a percentage of total leukocytes. The FCS files from evaluable specimens of the original LST clinical trial were retrospectively analyzed using BD OneFlow LST template v2.0.

Aims: The objective of this study was to regress the FCS files from all the evaluable specimens previously collected using LST template v1.0 in the original clinical study to demonstrate equivalency between the investigational BD OneFlow LST system and the comparator EF liquid reagent system on a BD FACSCanto II flow cytometer with the 4-2H-2V CE-IVD configuration and LST template v1.0.

Methods: The FCS files using LST v1.0 template from the original clinical study included de-identified remnant peripheral blood (n=123), bone marrow (n=53), and lymph node (n=31) specimens from patients and healthy donors. Specimens

haematologica | 2017; 102(s2) | 717

Madrid, Spain, June 22 – 25, 2017
were collected in EDTA or heparin anticoagulants or PBS (for lymph nodes) at three external study sites. Informed consent was not required in the clinic study. All specimens in the original study were simultaneously stained with investigational BD OneFlow LST and comparator EF liquid reagents within 24 hours of collection and were acquired within 60 minutes of staining. In the current study, analyses were performed on a BD FACSCanto II instrument using LST v2.0 template and BD FACSDiva software v6.0.1. Flow cytometry plots were categorized as normal or follow-up needed. If follow-up was needed, specimens were categorized as B-, T-, NK-, or other-cell lineage. Overall agreement, negative agreement, and positive agreement, along with their one-sided lower 95% confidence limits, were calculated. For quantitative (percent) comparison of defined cell populations, Deming regression (slope and intercept analysis) was performed between the BD OneFlow method and the EF method. Results: The BD OneFlow LST system compared to the EF system gave 100% (207 of 207) overall agreement (lower 95% CI: 98.6%) in delineating patients into normal (no follow-up) or follow-up, and 100% overall agreement in identifying B-, T-, and NK-cell lineages (lower 95% CI: 98.4%). There was 100% positive agreement and 100% negative agreement between BD OneFlow and EF for follow-up vs no-follow-up (normal) and for all cell lineages from specimens that required follow-up. Furthermore, compared to the BD OneFlow LST system, the BD OneFlow LST system met the acceptance criteria for the quantitation (Deming regression) for the defined cell populations.

Summary/Conclusions: The multisite performance evaluation of the BD OneFlow LST system and the comparator EF liquid reagent system was concordant in identifying abnormal from normal mature populations in patients with CLPDs. BD OneFlow LST is fit for In Vitro Diagnostic Use, CE Marked to the European In Vitro Diagnostic Medical Device Directive 98/79/EC: 23-19566-00.

PB1789 IMMUNOGLOBULIN HEAVY/LIGHT CHAIN ASSAY DETECT IMMUNE DYSREGULATION IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA


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Background: Chronic lymphocytic Leukemia (CLL) is frequently accompanied by immune dysregulation. Hypogammaglobulinemia is the most important associated immune defect and the three classes of immunoglobulins (IgA, M and G) are involved. Recently, a novel assay for detecting heavy/light chain (heavy/light) and their ratios has been described (HLC), which improves immunoglobulin detection and monitoring in plasma-cell dyscrasias by quantitating the different light chain types of each immunoglobulin class. The frequency and biological role of this assay has as yet not been studied in CLL.

Aims: To study the frequency of abnormal Heavy Light chain assay, in CLL patients.

Methods: This is an observational, multi-center study performed in collaboration with the Israeli CLL Study Group involving 10 medical centers in Israel. The cohort included patients with CLL as well as healthy volunteers. All patients studied had complete clinical database available and all medical records were examined and then summarized. Serum samples were analyzed for levels of: IgG1, IgG2, IgG3, IgG4, IgA kappa, IgA lambda, IgM kappa, IgM lambda and Free light chain: kappa (K) and lambda (L), ratio of K/L and calculation of ratios of monoclonal/polyclonal immunoglobulin (HLC ratio).

Results: The total cohort consisted of 126 “treatment - naive”, patients with CLL and 26 healthy volunteers. Median age was 64 years, 64% were males and 78% had Binet stage A, while 19% and 3% were stages B or C respectively. Significant differences in immunoglobulin levels with HLC ratio were noted between patients and controls (p < 0.0001). Abnormal HLC ratios were observed in 17% of patients (15% (p = 0.005) and 2% (p = 0.023)) and 8% (p = 0.005) and 2% (p = 0.023) respectively. There were higher levels of kappa heavy light chain (HLC ratio) in patients compared to controls (p = 0.0001 and 0.0001 respectively). The kappa(+) and lambda(-)(+) patients were categorized as B- and T- cell. Overall agreement, negative agreement, and positive agreement, along with their one-sided lower 95% confidence limits, were calculated. For quantitative (percent) comparison of defined cell populations, Deming regression (slope and intercept analysis) was performed between the BD OneFlow method and the EF method. Results: The BD OneFlow LST system compared to the EF system gave 100% (207 of 207) overall agreement (lower 95% CI: 98.6%) in delineating patients into normal (no follow-up) or follow-up, and 100% overall agreement in identifying B-, T-, and NK-cell lineages (lower 95% CI: 98.4%). There was 100% positive agreement and 100% negative agreement between BD OneFlow and EF for follow-up vs no-follow-up (normal) and for all cell lineages from specimens that required follow-up. Furthermore, compared to the BD OneFlow LST system, the BD OneFlow LST system met the acceptance criteria for the quantitation (Deming regression) for the defined cell populations.

Summary/Conclusions: The multisite performance evaluation of the BD OneFlow LST system and the comparator EF liquid reagent system was concordant in identifying abnormal from normal mature populations in patients with CLPDs. BD OneFlow LST is fit for In Vitro Diagnostic Use, CE Marked to the European In Vitro Diagnostic Medical Device Directive 98/79/EC: 23-19566-00.
on distribution of response in clinical trials. Data on disease progression and mortality was provided by the treating oncologist/hematologist. PFS and OS were compared using univariate and multivariate Cox proportional analyses between the CR and non-CR cohorts (OS multivariate analyses were not conducted due to the small number of events). An additional analysis was conducted to examine the benefits of achieving MRD- versus not achieving MRD- among patients who achieved CR or PR.

Results: Data was collected on 330 CLL patients, including 179 patients in the CR cohort and 151 patients in the non-CR cohort (120 patients with PR, 25 with SD, and 6 with PD). Most patients were male, in their early sixties, and had an ECOG status of 0/1 at the time of initiating first-line therapy. The median observation period was approximately 30 months. There were 43 (26%) patients in the CR cohort and 75 (50%) patients in the non-CR cohort who progressed/died (Table 1). Patients in the non-CR cohort had an >2-fold higher hazard of progression/death (adjusted hazard ratio [HR]=2.30, p<0.05) and death (unadjusted HR=2.61, p<0.05) compared to patients in the CR cohort.

Among patients who achieved CR or PR, 84 patients achieved MRD- and 62 patients did not; 14 (17%) patients who achieved MRD- and 27 (44%) patients who did not achieve MRD- progressed/died. Patients who did not achieve MRD- had an over three-fold higher hazard of progression/death compared to patients who achieved MRD- (adjusted HR=3.75, p<0.05). No death events were observed among patients who achieved MRD- while 4 (6%) events were observed among those who did not achieve MRD-.

Summary/Conclusions: Findings from this real-world study suggest that achieving CR is associated with improved PFS and OS compared to patients who do not achieve CR. Furthermore, significantly better outcomes were observed among those who achieved MRD- compared to those who did not achieve MRD- but still achieved CR or PR. This suggests that deep response may be an important clinical parameter to consider in the treatment of CLL.

PB1793

COMPARATIVE ANALYSIS OF INTERNATIONAL PROGNOSTIC INDEX FOR CHRONIC LYMPHOCYTIC LEUKEMIA, PROGRESSION-RISK SCORE AND MD ANDERSON CANCER CENTER 2011 SCORE: REAL WORLD DATA FROM A SINGLE INSTITUTION

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Background: In recent times, several powerful prognostic scores have been developed in order to predict to first treatment (TTFT) and overall survival (OS) of chronic lymphocytic leukemia (CLL) patients. The International prognostic index for chronic lymphocytic leukemia (CLL-IPI) developed by The International CLL-IPI working group was found to predict OS and TTFT, while the rest of two scores- progression-risk score (PRS) and MD Anderson Cancer Center Score 2011 (MDACC 2011) have been developed for prediction of TTFT in early stage CLL patients.

Aims: The aim of this study was to compare CLL-IPI, PRS and MDACC 2011 prognostic scores based on their impact on TTFT, treatment response (TR), progression-free survival (PFS) and OS of 54 treated CLL patients.

Methods: We retrospectively analyzed data from 54 consecutive CLL patients diagnosed and treated at Clinic for Hematology, Clinical Center of Serbia from 2003 to 2013. Blood samples were prospectively collected and analyzed for biological and molecular features (IGHV, FISH and TP53), as well as standard laboratory parameters. The three scores were retrospectively calculated using formulas from the original articles (International CLL-IPI working group, Lancet Oncol 2016, for CLL-IPI; Gentile et al, Leukemia 2016, for PRS; and Wierda et al, J Clin Oncol 2011, for MDACC 2011 score), and, than, correlated with TTFT, TR, PFS and OS of patients from the studied cohort.

Results: Median age at diagnosis was 57 years (range 38-75). All patients were treated with fludarabin-based chemotherapy, 45 (83%) in the first treatment line. Overall response rate to the first line therapy was 81%, equally distributed on complete and partial responses. Most of the patients (42, 78%) relapsed during the follow up. At the time of the last follow up 14 (26%) patients were still alive, 35 (65%) were dead, and 5 (9%) were lost to follow up. Median overall survival was 76 months. Lower score values for all the three scoring systems were predictive of better TTFT, PFS and OS. CLL-IPI and PRS were identified as significant predictors of TTFT (p=0.003, RR=1.4, 95%CI 1.1-1.7 and p=0.019, RR=1.4, 95%CI 1.1-1.9, respectively), while MDACC 2011 was of borderline significance (p=0.052). In the multivariable analysis PRS emerged as the most significant predictor of TTFT among the three examined scores (p=0.041, RR=1.35, 95%CI 1.01-1.81). Regarding TR, only PRS appeared to have borderline statistical significance (p=0.052), showing that patients with lower score value may achieve better TR. Lower CLL-IPI can predict longer PFS after the first line treatment (p=0.07, RR=1.7, 95%CI 1.2-2.57), as well as PRS (p=0.039, RR=1.35, 95%CI 1.03-1.75) and MDACC 2011 (p=0.005 for all). Cox regression analysis revealed that CLL-IPI and PRS are significant predictors of TTFT (p=0.003, RR=1.4, 95%CI 1.1-1.7 and p=0.019, RR=1.4, 95%CI 1.1-1.9, respectively), while MDACC 2011 was of borderline significance (p=0.052). In the multivariable analysis PRS emerged as the most significant predictor of TTFT among the three examined scores (p=0.041, RR=1.35, 95%CI 1.01-1.81). Regarding TR, only PRS appeared to have borderline statistical significance (p=0.052), showing that patients with lower score value may achieve better TR. Lower CLL-IPI can predict longer PFS after the first line treatment (p=0.007, RR=1.7, 95%CI 1.2-2.57), as well as PRS (p=0.039, RR=1.35, 95%CI 1.03-1.75) and MDACC 2011 (p=0.005 for all). Cox regression analysis confirmed PRS to have the strongest predictive value of all the three scores regarding duration of PFS (p=0.039, RR=1.8, 95%CI 1.02-3.1). Furthermore, CLL-IPI and PRS were found to be significant predictors of OS (p=0.005, RR=1.4, 95%CI 1.1-1.8 and p=0.037, RR=1.5, 95%CI 1.1-2.4), respectively. MDACC 2011 has not shown to have influence on PFS. Multivariable analysis confirmed PRS to have the strongest predictive value of all the three scores regarding duration of PFS (p=0.039, RR=1.8, 95%CI 1.02-3.1). Furthermore, CLL-IPI and PRS were found to be significant predictors of OS (p=0.005, RR=1.4, 95%CI 1.1-1.8 and p=0.037, RR=1.5, 95%CI 1.1-2.4), respectively.

Summary/Conclusions: CLL-IPI and PRS were identified as significant predictors of TTFT, as well as of duration of TR and OS. Further studies are warranted to confirm these findings.
MULTISITE PERFORMANCE EVALUATION STUDY OF THE BD ONEFLOW B-CELL CHRONIC LYMPHOPROLIFERATIVE DISORDERS T1 (B-CLPD T1) PANEL

Background: The BD OneFlow solution for B-cell chronic lymphoproliferative diseases (B-CLPDs) incorporates a standardized flow cytometry approach based on the EuroFlow (EF) Consortium liquid reagent system. The BD OneFlow solution enables reproducible identification and discrimination of distinct cell populations by combining standardized assays, setup reagents, and protocols. The previously launched BD OneFlow LST (Lymphocyte Screening Test) is intended for flow-cytometric immunophenotyping of normal (no follow-up required) and aberrant (follow-up required) mature lymphocyte populations of B, T, and NK lineages in specimens from patients with hematological disorders. The BD OneFlow B-CLPD T1 is being developed to work in conjunction with BD OneFlow LST for the immunophenotyping of B cells and distinguishing chronic lymphocytic leukemia (CLL) from other B-CLPDs such as atypical CLL, follicular cell lymphoma, mantle cell lymphoma, etc.

Aims: The objective of this study was to demonstrate equivalency (accuracy) between the investigational BD OneFlow LST and BD OneFlow B-CLPD T1 system and the corresponding comparator EF liquid reagent system on the BD FACS Canto II flow cytometer using BD FACSDiva software.

Methods: De-identified remnant peripheral blood (PB) (n=70) and bone marrow (BM) (n=31) patient specimens were collected in EDTA or heparin anticoagulants at four external study sites and tested within 26 hours of draw. Informed consent was not required in this clinical study. Specimens were stained with BD OneFlow LST in combination with OneFlow B-CLPD T1 tubes and comparator EF liquid reagents. Acquisition and analysis were performed on a BD FACS Canto II instrument using BD OneFlow LST and B-CLPD T1 templates in BD FACSDiva software v8.0.1. Categorization of samples with abnormal B-cell populations into CLL (typical) or other B-CLPDs, overall agreement, negative agreement, and positive agreement, along with their one-sided lower 95% confidence limits, were calculated. For qualitative categorization of relative fluorescence intensity (RFI) for membrane antigens of the aberrant cell populations, overall agreement with one-sided lower 95% confidence limits was calculated.

Results: All evaluable specimens were identified by the OneFlow LST as having B-cell populations requiring follow-up by both methods. Compared to the EF system, the BD OneFlow LST in combination with the BD OneFlow B-CLPD T1 system had 100% (101 of 101) overall agreement in classifying patients as having CLL (54 of 54 concordant) and in identifying patients with other B-CLPD diseases (47 out of 47 concordant) with a lower 95% CI of the overall agreement of 97.4%. The BD OneFlow B-CLPD T1 system, compared to the EF system, gave 100% (101 of 101) concordant agreement for the qualitative assessment of relative RFI for membrane antigens on the aberrant cell populations, overall agreement with one-sided lower 95% confidence limits was calculated.

Summary/Conclusions: The multisite performance evaluation between the BD OneFlow system (LST and B-CLPD T1) and the comparator EF liquid reagent system in distinct geographic areas and microenvironments are required to validate our findings and discard or confirm the potential role of some antigen-binding BCR specificities contributing to clonal evolution.
Background: Constitutive activation of B-cell receptor signalling appears to be essential for the proliferation of malignant B cells. Bruton's tyrosine kinase (BTK) has been identified as an essential component of the B-cell receptor signalling pathway. Ibrutinib is an orally administered BTK inhibitor that antagonises B cell receptor, chemokine & integrin mediated signalling.

Aims: We report our experience of using ibrutinib to treat relapsed/refractory B-cell chronic lymphocytic leukaemia (B-CLL) and mantle cell lymphoma (MCL) in a busy U.K. District General Hospital (DGH) serving a population of 600,000.

Methods: 26 patients were commenced on ibrutinib for relapsed/refractory B-CLL or MCL between August 2014 & December 2016. 16 patients had B-CLL and 10 patients had MCL. Patients with B-CLL were commenced on 420mg daily; those with MCL received 540mg daily. The median age at which ibrutinib was commenced was 71.1 years (range 50-85). The median age of patients with B-CLL was 71.1 years (range 50-80) and for MCL was 71.6 years (range 54-85). The median number of prior lines of therapy decreased over the time period from 3.2 in 2014 to 1.2 in 2016. The mean interval between diagnosis and first-line therapy for patients with B-CLL was 6.4 years (range 0.3-35.3) and 6.3 years (range 0.2-31.1) for MCL. The average number of co-morbidities in both groups was similar: 1.4 in B-CLL and 1.5 in MCL. After May 2015 all patients received aciclovir and co-trimoxazole prophylaxis. Response to ibrutinib was assessed by clinical examination and blood results; imaging and bone marrow examination were conducted at the clinician’s discretion.

Results: The median follow up was 15.5 months for B-CLL patients and 8 months for MCL patients. The median survival of all patients who did not receive anti-viral and pneumocystis prophylaxis was 5 months and the median survival for those who did receive prophylaxis was not reached (p<0.0001). The median survival of patients with MCL was 8 months. The median survival of those more than 1 year after first-line chemotherapy treatment was 17 months; the median survival in those who had received just one prior line of treatment was not reached (p=0.0085). In the B-CLL cohort there was no difference in survival between those with and without 17p/p53 deletion. 11/28 patients experienced side effects; 8 had grade 1 and 2 side effects (diarrhoea, drug rash, cardiac arrhythmias) which were easily controlled. 3 patients had grade 4 side effects (1 severe arthropathy, 2 intracranial haemorrhage - one of which was fatal). 4 of the 16 (25%) with B-CLL and 5 of the 10 (50%) with MCL died during the period of follow-up. Causes of death were: intra-cerebral haemorrhage (1), unrelated cancer (1), disease progression (2), disease progression+sepsis (2), sepsis alone (3). Of the remaining 17 patients, 14 continue to receive ibrutinib, 2 (B-CLL) were switched to idelalisib+Rituximab (for grade 4 toxicity) & 1 went on to have an allogeneic transplant (MCL).

Summary/Conclusions: Though our cohort of patients is small, our experience shows that the use of prophylaxis with co-trimoxazole and aciclovir is associated with improved overall survival. Moreover, patients who received fewer lines of prior treatment had a better survival. Patients with 17p/p53 deleted B-CLL responded as well those without a deletion. Ibrutinib is a very effective therapeutic option in patients with relapsed CLL and MCL.
LYMPHOCYTIC LEUKAEMIA TREATED WITH IBRUTINIB

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Background:
Chronic lymphocytic leukaemia (CLL) is characterised by frequent co-existent infectious complications. They stem from, among other things, hypogammaglobulinaemia, which is connected with CLL, and correlates with the disease duration and severity, as well as T-lymphocyte function disorders. The application of innovative therapies (chemoimmunotherapy) on the one hand facilitates considerable improvements in treatment outcomes and on the other hand it increases the risk of life-threatening infectious complications. The introduction of a new drug, ibrutinib (Bruton’s kinase inhibitor), has created a unique and promising opportunity to treat CLL patients, especially those with progranulocytically unfavourable genetic aberrations (del17p), or in the case of whom previous chemotherapies have failed to give satisfying results. Previous observations indicate the risk of side effects (e.g. bleeding, infectious complications, heart rhythm disorders) which might sometimes limit the applicability of ibrutinib in some CLL patients.

Aims:
The aim of this paper was to evaluate the risk of infectious complications in persons with CLL, and to determine potential correlations between possible infectious complications and selected clinical, morphological and biochemical parameters.

Methods:
The study comprised 43 CLL patients aged 48-82 years (average age 67 years), 18 women and 25 men. At the beginning of the ibrutinib therapy the patient’s disease was at the 2-4 clinical stage, according to Rai et al. Usually they were individuals who had received a couple of previous chemotherapies (from 1 to 7) which contained, inter alia, purine analogues, and the monoclonal antibodies (rituximab, alemtuzumab, ofatumumab). Ibrutinib was administered at a dose of 420 mg/d.

Results:
Infectious complications were observed in 16 patients (37.2%). These included, for example, upper respiratory tract infection, bronchitis, pneumonia, urinary-tract infections, pharyngitis. The conducted analysis showed a statistically significant correlation between the concentration of IgM in the blood serum (before ibrutinib administration) and infectious complications during these therapy (p<0.05). The average IgM concentration in patients with complications was considerably lower when compared to people who did not experience any complications. The patients (n=3, 6.98%) with complications at the moment of the CLL diagnosis also had them during the ibrutinib treatment. This phenomenon was confirmed in 13 patients (33%) in the other group. The correlation was borderline significant (p=0.09). Infectious complications were observed more frequently in the patients with 3-4 stage CLL (according to Rai et al.) than in the individuals at the less-advanced clinical stages of the disease (0-2), and this correlation also showed borderline significance (p=0.08). No significant correlation was detected between the risk of infectious complications and earlier therapy with purine analogues and neutropenic episodes during the ibrutinib therapy.

Summary/Conclusions:
Ibrutinib is considered to be a real breakthrough in CLL treatment; but it has to be borne in mind that the drug gives possible side effects which might occur during therapy. They include infectious complications which are among the main causes of death in this group of patients. The results obtained by us indicate that the risk of infection during ibrutinib therapy relates mainly to patients with low IgM concentration in the blood serum and at more advanced clinical stages of the disease. In this case the occurrence of previous complications (before ibrutinib administration) is also relevant. We are aware of the limitations of our work related to the small number of patients. Yet, even at this stage, it is possible to select CLL patients with increased risk of such usually life-threatening complications.

PB1800
INFECTIONOUS COMPLICATIONS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA TREATED WITH IBRUTINIB

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Background:
Monoclonal B-cell lymphocytosis (MBL) is a recently recognized entity characterized by the presence, in the peripheral blood, of a monoclonal B-cell population lower than 5000/µl, in the absence of any type of clinical features. MBL clones may have: a) chronic lymphocytic leukaemia (CLL-like) phenotype (CD5+, CD19+, CD23+, CD20 dim); b) atypical CLL phenotype (CD5+, CD19+, CD23- or CD20 bright); c) non-CLL phenotype (CD5-). MBL can be also distinguished in “low-count” (<500/µl) and “high-count” (>500/µl) subtypes. High-count MBL frequently shows typical CLL phenotypic/genetic features and require adequate follow-up in order to detect their possible evolution into symptomatic CLL. MBL showing a clonal B-cell count higher than 1000-1500/µl are usually defined “clinical” MBL. Using highly sensitive (i.e. >6 colors and >500000 events) flow cytometry approaches, CLL-like MBL clones have been found at a frequency of 7-12% in healthy subjects, showing, however, very low median counts of clonal B-cell (10-170/µl), with only 0.14% being clinical MBL. Though several studies have described the association between CLL and various types of neoplastic disorders, only few data exist about the risk of non-hematologic cancer in individuals with MBL, in particular, no association between MBL and prostate cancer (PC) has been so far reported.

Aims:
To study prospectively the frequency of CLL-like MBL clones in patients affected by PC compared to healthy males of the same ages, after our previous observational study of an apparently increased MBL incidence at baseline in a cohort of patients with PC originally studied to detect lymphocyte abnormalities possibly induced by radiotherapy (RT).

Methods:
We enrolled 34 consecutive patients affected by PC (mean age 74 years, range 58-91), naïve for chemotherapy (sixteen previously treated with hormone-therapy). All patients were planned to receive whole-pelvis RT with radical (n. 23) or salvage (n. 11) intent. Fifty-four healthy males (mean age 71 years, range 58-91) represented the control group. Immunophenotypic analysis of peripheral lymphocytes before RT was performed by BD FacsCanto II flow cytometer, using a 5-6 colors approach and the following antibody combinations: CD19 FITC/CD5 PE/CD45 PerCP/Cy5.5/CD20 PC9/Cy7/CD54 APC/C45 APC-Cy7. For each sample, 100000 events were collected. CD45+ lymphocytes were gated on CD45 vs SSC dot plot, then B cells were isolated by gating on CD19 and CD19+ CD5+ cells were interrogated for intensity of CD20. Finally, CD19+ CD5+ CD20dim selected population was analyzed for light chain clonality and CD23 expression.

Results:
Median (range) absolute counts of white blood cells (WBC), total lymphocytes and B-cells, as well as absolute single values of MBL clones are reported in Table 1. In PC patients we found 3 MBL (8.8%), two of which were “high-count” MBL (1.8%) was detected, showing a very small clone (8 cells/µl). Such a difference was not statistically significant (p=0.2).

Table 1.

Summary/Conclusions: The preliminary results of our prospective study, performed using a routine, not highly sensitive flow cytometry approach, highlight a possible association between (clinical?) MBL and PC, never described before and probably warranting further investigation in a larger number of patients.

722 | haematologica | 2017; 102(s2)
Chronic myeloid leukemia - Biology

PB1802
IDENTIFICATION OF NOVEL MUTATIONS IN CANCER-RELATED GENES IN HUMAN ERYTHROLEUKEMIA K562 CELL LINE BY NEXT-GENERATION SEQUENCING
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Background: Chronic myeloid leukemia (CML) is a clonal hematopoietic stem cell disorder characterized by reciprocal chromosome translocation t(9;22)(q34;q11), resulting in the formation of the BCR-ABL fusion oncogene. One of the most widely used CML in vitro model is the K562 cell line, a positive human human erythroleukemia cell line derived from a female patient with CML in blastic phase (CML-BP) and representing an important tool for the studies of malignant hematopoiesis in last decades. Although K562 karyotype was described several times, detailed genomic analysis of this cell line is not yet available and to our best knowledge there are no publications yet describing complex genomic landscape of K562 cells.

Aims: The aim of our study was to determine the mutational landscape of K562 cell line using next-generation sequencing (NGS). Additionally classical fluorescence in situ hybridization (FISH) with BCR and ABL1 probes was performed to confirm cytogenetics.

Methods: The K562 cell line was purchased from DSMZ (Braunschweig, Germany). We analyzed almost 1300 genes implicated in human cancer using custom designed capture (SeqCap EZ, NimbleGen, Roche) followed by high-throughput sequencing on Illumina HiSeq 1500. Common variants (>1%) gathered in 500 and 1000 genomes projects and our internal exome database were filtered out and the subsequent analysis was focused on putative protein damaging variants with the frequency in the database from NHLBI GO exome sequencing project less or equal to 0.01. We used different bioinformatic tools for variant effect prediction (eg. PolyPhen-2, SIFT, IntOGen). Mutations were confirmed with Sanger sequencing. FISH was performed using commercially available probes (Vysis, Abbott, USA), that identifies BCR-ABL1/ABL1-BCR fusion genes.

Results: Sequencing and bioinformatics analysis revealed 88 variants with potential biological significance. We detected Q136fs*13 mutation in TP53, which has already been described in K562 cell line previously by ATCC, but we have also identified several new mutations in genes involved in tumorigenesis and drug resistance (Table 1). Moreover, cytogenetic analysis showed both multiplication of the BCR and ABL1 genes and amplification of the BCR-ABL1 fusion gene (Ph chromosome is present in at least four additional copies).

Table 1. Selected prominent mutations identified in K562 cells.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>NCIH Reference</th>
<th>Gene</th>
<th>Impact</th>
<th>Nearest Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53 c.410T&gt;GT (p.T137X)</td>
<td>NM_001261442.2</td>
<td>TP53</td>
<td>frameshift</td>
<td>NM_000031.3</td>
</tr>
<tr>
<td>ASXL1 c.3773G&gt;T</td>
<td>NM_03239.5</td>
<td>ASXL1</td>
<td>missense</td>
<td>NM_03239.5</td>
</tr>
<tr>
<td>RBCX2 c.2622G&gt;T</td>
<td>NM_02256.5</td>
<td>RBCX2</td>
<td>nonsense</td>
<td>NM_02256.5</td>
</tr>
<tr>
<td>AKT1 c.3037T&gt;G</td>
<td>NM_002892812</td>
<td>AKT1</td>
<td>missense</td>
<td>NM_002892812</td>
</tr>
<tr>
<td>BRCA2 c.4690G&gt;A</td>
<td>NM_00023.3</td>
<td>BRCA2</td>
<td>missense</td>
<td>NM_00023.3</td>
</tr>
</tbody>
</table>

Summary/Conclusions: We describe several new mutations in such genes as ASXL1, BRCA1 or MLH1 in one of the most frequently used cell line in leukemia research, K562 erythroleukemia. Our results confirm high level of genomic instability in the blastic phase of CML represented by the K562 cell line and add new, valuable information for researchers who want to employ this cell line. The awareness of the genomic aberrations present in the K562 erythroleukemia cell line is essential for further studies as those aberrations may have a significant impact on the observed results.

PB1803
INVESTIGATION OF POLYMORPHISMS RELATED TO MIR-608 IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA
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Background: Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by the expression of the BCR-ABL oncoprotein, which is essential for the pathogenesis of the disease. Imatinib, an ATP-competitive selective inhibitor of BCR-ABL, exhibits unprecedented efficacy for the treatment of CML. Several cellular and genetic mechanisms of imatinib resistance have been proposed, including overexpression of the BCR-ABL gene, the tyrosine kinase domain mutations, pharmacokinetic and pharmacodynamic factors.

Aims: The purpose of this study was to investigate miRNA-608 role in response to therapy with tyrosine kinase inhibitors (Imatinib). In this study, we analyzed rs9762 SNP located in a miRNA-608 binding site of 3’UTR of BCR-ABL gene and rs4919510 SNP in the mature sequence of miR-608 in CML patients with different response to tyrosine kinase inhibitor therapy. These polymorphisms disrupt the negative effect of mir-608 on its target BCR-ABL gene. Up-regulation of the BCR-ABL gene can develop resistance to ABL TKI and contribute to the treatment outcome.

Methods: In our study 76 CML patients at the age of 15–65 were involved. Genomic DNA was extracted from peripheral blood leukocytes by standard phenol-chloroform method. Genotyping was performed by the PCRRFLP technique.

Results: Combination of genotypes affecting mir-608-BCR-ABL1 interaction revealed different response to therapy. MiR-608 binding site G>G allele (*GG in miRNA itself) was associated with 81% in CML patients with uneffective therapy. We suggest that miR-608 could possess oncosuppressing activity as miR-203 but it should be confirmed by further experiments.

Summary/Conclusions: miRNAs could be a perspective tool for therapy and polymorphisms affecting its regulation should also be considered.
**PB1805**

**Fluorescence in situ hybridization signal patterns and intrachromosomal BCR-ABL1 amplification analysis in imatinib-resistant chronic myelogenous leukemia patients using trio-color dual fusion probe.**

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**Background:** Conventional cytogenetics is a common modality for tyrosine kinase inhibitor (TKI) response assessment in chronic myelogenous leukemia (CML) patients. There is no consensus regarding the use of conventional bone marrow (BM) cytogenetics or peripheral blood (PB) interphase fluorescence in situ hybridization (I-FISH) during follow-up. The routine dual colour FISH probes are less sensitive to reliably identify der(9) deletions during follow-up. BCR/ABL/ASS1 tri-colour dual fusion (TCDF) probe is highly sensitive and specific in identifying der(9) deletions.

**Aims:** Our aim was to identify the I-FISH fusion patterns of BCR/ABL/ASS1 TCDF probe and correlate the patterns with patient-specific molecular genetic parameters.

**Methods:** This was an ethically approved study conducted at a government-funded tertiary care institute. From January 2015 to June 2016, PB I-FISH analysis was performed on European LeukemiaNet defined imatinib-resistant CML patients using BCR/ABL/ASS1 TCDF probe (Abbott Laboratories, Abbott Park, Illinois, USA). The residual BCR-ABL1 transcript load was monitored in international scale (BCR-ABL1 IS) using an automated cartridge-based GenExpert system (Cepheid, Sunnyvale, CA, USA).

**Results:** On analyzing 37 adult patients, all had residual Philadelphia (Ph) chromosome (100%). Classic Ph fusion pattern was seen in 33 (89%), derivative chromosome 9[der(9)] deletions in 25 (67.5%) and supernumerary Ph chromosomes in 11 (30%) patients. Coexistence of classical fusion and der(9) deletions were seen in 21 patients (57%), whereas 8 patients (22%) had a mutual existence of classical fusion, der(9) deletions and supernumerary Ph chromosomes. None had Ph amplification. Figure 1 demonstrates the I-FISH patterns seen in a 43-year-old male diagnosed with CML-CP and had progressed to blast crisis at his 72nd month of imatinib therapy. In this Figure red, yellow and white arrows indicate blast cells without Ph chromosome, Ph+ blast with a loss of residual ABL1 on der(9)classical and random signal overlap, respectively. A mean (± S.D) of 29% (± 30%) and 18% (± 17) der(9) deleted cells were seen amongst patients with b2a2 and b3a2 BCR-ABL1 transcript types, respectively and this difference was statistically significant (p<0.008). There was also a significant difference in the disease transformation status according to the percentage of der(9) deleted cells (p=0.03). In this regard, patients with progressive disease (accelerated phase/blast crisis progression) had a mean (± S.D) of 47% (± 35) der(9) deleted cells in comparison to 19% (± 20) such cells in patients without disease transformation. In addition, patients with Ph duplication/triplication had a mean (± S.D) BCR-ABL1 IS levels of 49.478% (± 40.184), in comparison to BCR-ABL1 IS levels of 16.00% (± 19.993) in patients without these anomalies and this difference was also statistically significant (p=0.029).

**Summary/Conclusions:** Our work would be an appropriate reference material for I-FISH signal interpretation using BCR/ABL/ASS1 TCDF probe. We have demonstrated a high frequency of der(9) deletions, clonal heterogeneity and absence of BCR-ABL1 amplification in an imatinib-resistant Indian CML cohort. For the first time, a significant association of der(9) deleted cell percentage with b2a2 transcript type and disease transformation status has been identified and the same has to be tested in a larger cohort.

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**PB1806**

**Are you actually suspecting a chronic myeloid leukemia when ordering a BCR/ABL RT-PCR?**

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**Background:** Chronic myeloid leukaemia (CML) is a myeloproliferative neoplasm (MPN). It is characterized by a reciprocal (t9;22) (q34;11.2) resulting in the fusion oncogene BCR/ABL1 in a homoeotic stem cell. Clinical features are absent in nearly 20-40% of patients at diagnosis time. Hence, laboratory suspicion is crucial. Peripheral blood shows leukocytosis with left shift and "myelocyte bulge", absolute eosinophilia, and absolute basophilia invariably present1-3. The demonstration of the Philadelphia (Ph) chromosome with cytogenetic analysis, or BCR/ABL fusion gene by qRT-PCR will confirm the diagnosis (typical CML).

**Aims:** In order to gain accuracy when BCR/ABL PCR is ordered, we review myeloproliferative hematimetric parameters, with special focus in basophilia, before performing molecular analysis.

**Methods:** We retrospectively reviewed 299 BCR-ABL PCR requests received at our laboratory between January 1, 2015 and January 1, 2017. 80% of the total requests were ordered by haematologists physicians, 13.46% by other medical specialties (11.5% internal medicine) and 7.7% from the laboratory. Complete blood cell count (CBC) were analysed by ADVIA 2120. Neutrophilia was defined in our laboratory as absolute neutrophil count of >7.7x10^9/L, and basophilia was defined as absolute basophil count of >0.2 x10^9/L. A total of 299 requests for PCR of BCR-ABL were reviewed by laboratory hematologists before performing the test. Clinical and hematimetric parameters, with special focus in basophilia, were examined when ordering BCR-ABL PCR. We performed 235 test (78.6%) and 64(21.4%) were considered inadequate according former criteria. qRT-PCR p210 was performed and if a negative result was obtained with high CML suspicion qRT-PCR p190 and qRT-PCR p230, such as cytogenetic studies were performed. The statistical analysis was performed with STATA.

**Results:** 235 BCR-ABL by PCR tests were performed and 24 (10.21%) resulted positive. 167 (71.06%) were placed for neutrophilia; 41 (17.87%) for thrombocytosis and 26 (11.07%) for other criteria (eosinophilia, monocytosis, splenomegaly or combined). Among 24 positive cases 100% presented basophilia at diagnostic time and 91.66% (22/24) presented basophilia and neutrophilia. Two cases without neutrophilia at diagnosis were CML with extreme thrombocytosis. We found 33 cases with basophilia among 235 patients. 24 cases (72.73%) were diagnosed of CML and 9(27.27%) resulted in other MPN Ph- or unclassifiable MPS/MDS neoplasm. Our results show that when CML is suspected, basophilia>0.3 x10^9/L has a 100% sensitivity and 95.75% specificity. ROC curve for basophilia as a screening test before performing BCR/ABL PCR is 0.984 (Figure 1).

**Figure 1.**

**Summary/Conclusions:** Our results show that basophilia should be carefully investigate when CML is suspected, with high sensibility (100%) and specificity (95.75%). In cases no CML with basophilia >0.3 x10^9/L, further investigation should be performed in order to diagnose a MPN Ph- or MDS/MPN. Even basophilia is well stablished as nearly universal in CML 1,3,4, this study reveals it is not always pursuse enough, when clinicians ask for a molecular study.

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**PB1807**

**BCR-ABL DEL C.1086-1270 (PR362FS*21) AND TKI RESISTANCE IN CML PATIENTS FROM RUSSIAN FEDERATION**

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**Background:** Data concerning the impact of BCR-ABL del. c.1086-1270 on TKI resistance in CML is still controversial. This mutation was first described by Curvo et al. (2008) and was thought to confer TKI resistance. However computer modeling performed by Meggyesi N. et al. (2012) revealed disruption of ATP binding site in mutated tyrosine kinase therefore abrogating enzymatic activity. Nevertheless pathogenic effect of BCR-ABL p.R362fs*21 could be attributed to the formation of heterodimer with "wild type" Bcr-Abl p210 as described by Poulikakos P.I. et al. (2011).

**Aims:** To assess the impact of BCR-ABL del. c.1086-1270 (p.R362fs*21) on TKI resistance in CML patients from Russian Federation.

**Methods:** 33 male and 49 female CML patients (age 24-80) with BCR-ABL transcript level >0.1% were included in the study. BCR-ABL del. c.1086-1270 was estimated by nested PCR followed by Sanger sequencing. Initial screening for deletions was performed by means of fragment analysis (Applied Biosystems 3130).

**Results:** 92 RNA (cDNA) samples isolated from peripheral blood of 82 CML patients were tested. BCR-ABL del. c.1086-1270 (p.R362fs*21) was found in 32 patients (39%). 15 out of 32 (47%) patients with deletion were TKI sensitive while 17 (55%) were TKI resistant. In one TKI resistant case BCR-ABL del. c.1086-1270 was accompanied by BCR-ABL c.844G>C p.E282Q point mutation not described so far (Figure 1). This mutation was found in BCR-ABL del. c.1086-1270 transcript only and was absent in "wild type" Bcr-Abl p210 transcript amplified from the same patient.

**Figure 1.**

**Summary/Conclusions:** BCR-ABL del. c.1086-1270 could be found in almost half of CML patients and have no evident impact on the induction of big molecular response in TKI sensitive cases. Our observation that independent c.844G>C p.E282Q point mutation expressed on the same BCR-ABL transcript with deletion c.1086-1270 (p.R362fs*21) being absent in "wild type" transcript strongly contradicts the hypothesis, that del. c.1086-1270 could be generated by alternative splicing of "wild type" BCR-ABL transcript.

**PB1808**

**PEROXIREDOXIN II ACTIVITY HAS IMPORTANT ROLES TO CONTROL ABL TYROSINE KINASE ACTIVITY IN STIS TREATED CML PATIENTS AND ITS POTENTIAL APPLICATION IN IMATINIB RESISTANCE**

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**Background:** Therapies targeting the redox environment such as over-expression of antioxidants or antioxidant treatment, could inhibit tumor cell growth even resistant cells. Bcr-Abl oncoprotein is known to induce high levels of intracellular ROS and its catalytic activity. Variable expression of antioxidant enzymes in leukemia, with limited studies with variable results so far. Altered redox biology in leukemia also has implications for therapeutic permeation.

**Aims:** We investigated the role of PRX II in CML primary cells at diagnosis and remission during signal transduction inhibitor (STI), and tested the same roles in Ph+ cell lines.

**Methods:** Three BCR-ABL1 positive cell lines with different resistance to TKI and generating IM-resistant K562 cells by chronic exposure of increasing concentrations of IM were compared with cell growth by MTT assay. BCR/ABL expression by western blot analysis, changes of intracellular ROS level and antioxidant enzymes such as peroxiredoxin (Prx) 1, 2, 3 using immunoblot assay according to different concentrations of IM between 0 to 10 μM in time dependent manner (24 hours/48 hours). We also repeatedly investigated the effects of IM therapy using PRXII overexpressed K562 cells by transfection.

**Results:** Three BCR-ABL1 positive cell lines showed significant change in cell viability. Intracellular ROS level, eradication of BCR/ABL oncogene and levels of Prx2 during IM treatment with different response each other in degree and pattern by IM exposure. The levels of BCR-ABL1 oncogene were slightly decreased in Prx2 overexpressed K562 cells. Moreover, Prx2 overexpressed K562 cells showed further down-regulation of Bcr-Abl oncoprotein by IM treatment.

**Summary/Conclusions:** Our findings may contribute to find a new pathway on which TKIs are working besides the mechanisms of ATP binding competitively, blocking the binding of ABL-kinase and substrate resulting apoptosis of Ph+ cells. In addition develop the new strategies to overcome the situation of the imatinib resistance. P10. BCR-ABL positive disease in the future. The importance of the roles of ROS and its PRX II, antioxidant enzymes in CML is further established by our work.

**PB1809**

**FUNCTIONAL CHARACTERISTICS OF ERYTHROID PROGENITOR CELLS OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH IMATINIB AND NILOTINIB**

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**Background:** It is believed that chronic myeloid leukemia arises as a result of myeloid progenitor cell malignancy. There are changing of proliferative activity in granulocyte-macrophage and erythroid hematopoiesis germs in patients bone marrow. Currently we don’t have definitive results regarding tyrosine kinase inhibitors influence on erythropoietic cell characteristics of patients with CML.

**Aims:** The aim of study was to determine functional characteristics of erythroid progenitor cells of patients with chronic leukemia treated with Imatinib and Nilotinib.

**Methods:** We studied 300 bone marrow samples from 75 patients: with initial diagnosis of CML (n=7), patients receiving drug imatinib (n=47) and patients who taking nilotinib (n=21). We provide studying of erythroid mononuclears in semisolid in vitro and in vivo cultures. For in vivo culture we used special gel capsule, allowing cytokines and growth factors of mouse body affect human mononuclear cells. For in vitro culture we added 20% fetal calf serum, 30 ng/ml erythropoietin, and 20 ng/ml minterleykin-6 and interleukin-9. Cultivation was provided 14 days, then counted the number of erythroid colonies and provided their morphological studies.

**Results:** The results showed that the increase of erythroid progenitor cells proliferation rates and the reduction of differentiation rates as a result of the parallel cultivation of patients’ bone marrow cells in vivo and in vitro happen irrespective of the presence of cytokines and growth factors in a normal microenvironment of these cultures. In addition, we showed that bone marrow cells of CML patients form erythroid colonies, while placed in the animals’ bone marrow form leukemiaous anemia. Moreover, correlative relationship was found between the number of erythroid colonies and the number of leukemic cells in the patients bone marrow. It was established that the acquisition of leukemia cell clones resistance to TKI is characterized by increased proliferative activity irrespective of soluble microenvironment factors as well as the culture medium in the erythropoietin presence.

**Summary/Conclusions:** The normal microenvironment factors not effect on the erythroid progenitor cell proliferation independence of the response to TKI therapy. This may explain the fact that we don’t have an increase the number of erythroid cells in patient bone marrow compared to culture in vitro. In addition, the ability of erythroid progenitor cells to form colonies in the absence of erythropoietin in culture can serve as an additional prognostic factor in the formation of resistance to TKI.

**PB1810**

**DEVELOPMENT OF FRAGMENT ANALYSIS MULTIPLEX-PCR METHOD TO DETECT TRANSCRIPTS OF BCR-ABL FUSION GENE IN CHRONIC MYELOID LEUKEMIA**

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**Background:** Chronic myeloid leukemia (CML) is a myeloproliferative, clonal and acquired hematological disease that is included within myeloproliferative neoplasms (WHO 2016). Its main characteristic is the presence (95% of the
case) of a small chromosome denominated Philadelphia chromosome, coming from the reciprocal translocation between chromosomes 9 and 22. Depending where the break-point occurs, different isoforms of the fusion gene BCR-ABL may appear. For the diagnosis of CML, detection of BCR-ABL rearrangement is crucial; and molecular biology techniques, such as RT-PCR, may be the only data at that point, but most current RT-PCR methods for detecting BCR-ABL are designed and optimized for detecting the major forms (e14a2 and in 32a2) without distinguishing between them. Characterization of the transcript is not necessary for the diagnosis but permits follow-up at the molecular level and differentiate between different BCR-ABL isoforms at the time of the CML diagnosis could be taken into account in future studies to investigate its role into the prognosis.

Aims: To develop a new multiplex RT-PCR method coupled to fragment analysis by capillary electrophoresis to identify different BCR-ABL isoforms: e13a3, e19a2, e1a4a3, e6a2, e1a3, e13a2, e14a2, e1a2 and e8a1.

Methods: 34 CML patients BCR-ABL positive by qRT-PCR and 1 negative control were selected for this study. First, we characterized our patients by means of molecular biology in January 2017. Results: BCR-ABL fusion RNAs were detected in all patients (34/34), on the other hand we did not detect BCR-ABL on the negative control. The main isoform identified was e14a2 (detected in 22 out of 34 patients, 64.7%). Twelve patients were positive for e13a2 BCR-ABL isoform (35.3%). Interestingly we identified 7 patients (20.5%) with co-expression of e14a2 and e13a2 isoforms, being in all these cases the e14a2 isoform mainly expressed.

Summary/Conclusions: RT-PCR combined with capillary electrophoresis is relevant as a novel technique for the detection of different isoforms of BCR-ABL and may be included as a BCR-ABL first screening. Quantification with qRT-PCR might only be done in positive samples. Unfortunately we could not detect any isoform besides the majority ones, due to the size of our cohort. Finally, our study validates previous studies on the main BCR-ABL isoforms (e14a2 and e13a2) percentage detected in CML patients.

PB1811
Abstract withdrawn.

PB1812
PDGF AND BDNF PLASMA LEVELS IN CML PATIENTS BEFORE AND AFTER INITIATION OF TKI THERAPY

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Background: Chronic myeloid leukemia (CML) is a malignant myeloproliferative neoplasm, which is characterized by 9;22(pq34.1;q11.2) translocation, also known as the Philadelphia chromosome (Ph). The resulting fusion gene BCR-ABL encodes a constitutively active tyrosine kinase that dictates the pathophysiology of CML. Tyrosine kinase inhibitors (TKIs) have been shown to efficiently inhibit not only the Bcr-Abl kinase, but also act on other cell surface tyrosine kinase receptors, such as the platelet-derived growth factor receptor (PDGFR). Similar receptors are vital in neurotrophin-mediated signaling pathways, for example TrkB receptor for brain-derived neurotrophic factor (BDNF). PDGF is a potent mitogen for cells of mesenchymal origin and plays a significant role in angiogenesis, a process which has recently been recognized as crucial for tumor growth and survival of neoplastic cells of the hematopoietic system. BDNF acts on certain neurons of the central nervous system and the peripheral nervous system and has a wide role in neuroprotection and neuroregeneration. However, the exact roles of PDGF, BDNF and their receptors in normal and malignant hematopoiesis remain unclear.

Aims: In this study, we aimed to investigate the levels of PDGF-AA and BDNF in plasma from CML patients and, where possible, to identify how TKI treatment affects these proteins levels.

Methods: Peripheral blood samples were obtained from newly diagnosed CML patients (n=5), CML patients treated with TKIs (n=5) and healthy controls (n=10). Informed consent was obtained from all subjects included in the study. Plasma PDGF-AA and BDNF levels were analyzed using Luminex technology with Human Neurodegenerative disease Panel 3 kit (Merck Millipore, Billerica, USA).

Results: We have observed that PDGF-AA levels were elevated in CML group (both before and during TKI treatment) compared to controls. Interestingly, we have also observed a lower level for newly diagnosed CML patients was not compared to TKI-receivers (p < 0.05). In case of BDNF, we have observed subtle changes between the tested groups: BDNF level in newly diagnosed CML subjects was lower compared to controls (p < 0.05), but in TKI-receivers the level was comparable to control group (p > 0.05). We have also tested one patient in a frequent time points (at diagnosis, 3 months with TKIs, 6 months with TKIs) for both PDGF-AA and BDNF - we have observed PDGF levels drop and BDNF rise with time.

Summary/Conclusions: In our study we have demonstrated that PDGF-AA and BDNF are feasible targets for plasma proteomic analysis in CML patients, both for studying the patterns of protein expression on leuc and also for identifying proteins differentially expressed before and during TKI treatment. We have shown that PDGF level drops down after TKI treatment, while on the opposite BDNF level in plasma raises with time in CML patients receiving TKIs. We have also monitored these proteins levels over time in the same patients, despite the fact this group is too small to draw meaningful conclusions. Further studies are required to elucidate the PDGF, BDNF and possibly other growth factors, neurotrophins and their receptors role in normal and malignant hematopoiesis.

PB1813
A CASE OF ATYPICAL CHRONIC MYELOID LEUKAEMIA WITH LATE DISCOVERY OF JAK2

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Background: Myeloproliferative neoplasms (MPN) include on the one hand chronic myeloid leukaemia defined by the presence of Philadelphia chromosome and BCR-ABL remodeling, and on the other hand MPN without Philadelphia chromosome (Polycythemia vera [PV], essential thrombocythemia [ET] and primary myelofibrosis [PMF]). V617F JAK2 mutation is the main recurring genetic abnormality in these pathologies (1). It can be found in 95% of PV and 50% of ET and PMF (2). The 2016 WHO classification makes no proposal of an entity either a V617F JAK2 mutation during treatment by tyrosine kinase for a BCR-ABL+CML; or a BCR-ABL+CML during treatment for a V617F JAK2+MPN (3,4,5,6,7). A very small number of patients showed coexistence of those two mutations (8).

Aims: We report a 62y old woman patient with chronic myeloid leukaemia with late discovery of JAK2.

Methods: Clinical presentation: A 62-year-old man with no notable medical history was admitted in 2009 for CML. After failure of first line treatment by Imatinib in 2009 (poor tolerance and incomplete molecular response), treatment by Nitrobin was initiated in 2012 allowing for, to this day, good molecular response despite poor digestive tolerance in the form of dyspepsia. Ever since, the patient's counts were stable with platelet count (PLT) at 726 × 10^9/L, WBC at 4.8 × 10^9/L and hemoglobin (Hb) at 13.1 g/L. In 2015, the patient was admitted in our department for digestive symp- toms with low Hb (9.8 g/dL) and PLT (100 x 10^9/L). Blood count whereas hemoglobin and platelets had normalized. To determine whether or not V617F JAK2 mutation was present at the time of CML diagnosis, a 2009 sample, in which JAK2 V617F had been estimated at less than 1%, was reanalyzed by means of molecular biology in January 2017. This exam found the mutation in quantities below the clinical significance threshold (1%). But this positivity, however small (0.19%), shows preexistence of the pathological clone.

Summary/Conclusions: This patient's case can be integrated in the series of cases described in 2013 by Park et al. (9) as it consists of V617F JAK2 positive ET onset during treatment for a BCR-ABL positive CML. The physiopathology of those two pathologies has not yet been genetically determined (8). Are those two independent pathologies or do they share a common tumoral clone? In this case JAK2 and BCR-ABL evolved in negative correlation and as such it is surmised that there were in fact two independent diseases, with two preexisting pathological clones at the time of the first diagnosis, treatment of the first pathology having been responsible for the proliferation of the second clone.
Figure 1.

**Chronic myeloid leukemia - Clinical**

**PB1814**

**E14A2 TRANSCRIPT IS ASSOCIATED WITH HIGHER PROBABILITY OF DURABLE TREATMENT FREE REMISSION IN CML PATIENTS**

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**Background:** TKIs discontinuation in CML-CP patients with deep molecular response (DMR) are feasible, safe and 40-60% of them maintain treatment free remission (TFR); sokal risk score and duration of TKI-therapy were significantly associated with molecular relapse, according to Euro-Ski and STIM1 trials. While it is known that patients with e14a2 achieve earlier, deeper and more durable responses compared to those with e13a2, few information is available on the influence of the type of bcr-abl transcript on TFR duration.

**Aims:** Here we describe our single center experience of TKI discontinuation in CML-CP patients with sustained DMR.

**Methods:** Bcr-abl transcripts were determined by RQ-PCR analysis performed in accordance with EAC protocol (Gabert et al, Leukemia 2003) and to the standards of the Italian national network Labnet. All 174 CML-CP patients presently followed at our institution according to ELN guidelines and treated with 1st or 2nd TKIs were analysed: 103 (59%) had e14a2 and 69 (40%) e13a2 transcript (in 2 pz bcr-abl were not detectable). Criteria for TKI discontinuation was sustained DMR (MR4 or better) for at least 2 years. After TKI withdrawal, RQ-PCR for BCR-ABL was performed every month during the first year and every 2 months thereafter. TKI treatment was reintroduced immediately if DMR loss occurred. TFR was defined as the time between the date of TKI cessation and the date of restarting treatment for DMR loss or, if TKI was not resumed, the date of the last contact.

**Results:** Forty-nine patients, 25 male and 24 female, discontinued TKI treatment. At the time of discontinuation median age was 63 years (43-85), median time from TKI start 113 months (30-172), median duration of sustained DMR 60 months (24-153). Sokal distribution was 49%, 29% and 20% for low, intermediate and high risk (one patient was not evaluable). Among our 174 patients 39% (40/103) of all e14a2 patients and 13% (9/69) of all e13a2 discontinued TKI (P 0.0002, chi square). Thirty-six patients discontinued imatinib (11 of them with previous INF treatment), 13 stopped nilotinib (8 in first line, 5 in second line treatment). Median follow up after treatment discontinuation was 19 months (3-76), including 31 patients with follow up > 12 months. Thirty (26%) patients lost DMR. Median time off-therapy for these patients was 3 months (2-8), and only 1 lost DMR after 6 months. Therapy was restarted in all 13 patients (2 in MR1, 4 in MR2, 7 in MR3), 10 achieved a second DMR after a median interval of 2 months (1-7); 2/13 patients are in M3 after 7 and 12 months, 1 patient is not yet evaluable. Ten out of 11 patients treated with INF before imatinib remained in TFR. Of note, the type of bcr-abl transcript was significantly linked to DMR loss: after TKI discontinuation, 32/40 e14a2 patients (80%) maintained DMR vs 9/13 e13a2 patients (42%) (p 0.03). After 12 months 78% (+/-6% CI95%) of e14a2 and 41,6 (+/-17% CI95%) of e13a2 patients were still in TFR (log-rank: P=0.033) (see Figure 1). Using multivariate analysis the type of bcr-abl transcript and previous INF treatment correlated with DMR loss (p 0.012 and p 0.033). One patient died during follow up in DMR for CML-unrelated cause.

**Figure 1.**
Summary/Conclusions: in e14a2 CML patients the probability of discontinuation of sustained DMR is significantly higher as compared with e13a2 patients. Moreover, after discontinuation, e14a2 have significantly lower probability of DMR loss than e13a2. These data confirm that e14a2 transcript is associated with a more favorable CML disease profile than e13a2 (Jain et al., Blood 2016); in addition they show that e14a2 is a favorable prognostic factor for TFR maintenance.

PB1815 COMPARATIVE ANALYSES OF NILOTINIB VS HIGH-DOSE IMATINIB VS SUSTAINING STANDARD-DOSE IMATINIB IN PATIENTS WITH CP CHRONIC MYELOID LEUKEMIA FOLLOWING SUSTAINED RESPONSE TO FIRST-LINE IMATINIB
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Background: Imatinib (IM) and its generic form are widely used as one of the standards of care for chronic phase (CP) chronic myeloid leukemia (CML). Although 7-year data by the IRIS demonstrated the long-term prognostic value of molecular response at specific time points, achieving major molecular response (MMR) at 18 months showed minimal event-free survival (EFS) benefit, compared with not achieving MMR but having complete cytogenetic response (CCyR). In addition, the best treatment for these patients remains less clear.

Aims: In this study, we investigated the efficacy of nilotinib (NIL) versus high-dose IM versus sustaining standard-dose IM for the patients with CCyR with suboptimal molecular response to first-line IM therapy.

Methods: Early CP CML patients who have achieved CCyR but not MMR after 18 months and 1st-line IM therapy at a daily dose of 400 mg were divided into the three treatment groups; nilotinib (NIL) 400 mg QD (800 mg/day; group 1) vs IM 400 mg QD (800 mg/day; group 2) vs IM 400mg QD (400mg/day; group 3). Group 1 and 2 patients were selected in the RE-NICE multicenter study, in which crossover to the alternate treatment arm was allowed for patients failing to achieve MMR at 12 months and for intolerant patients, and for patients who lost MMR at any time of treatment. Group 3 patients who have achieved CCyR but not NIL MMR after at least 18 months of first-line IM therapy were selected from the Asia CML Registry (ACR) database system with the same inclusion criteria of RE-NICE. The efficacy endpoints are MMR rate by 12 months and MMR rate and undetectable molecular residual disease (UMRD) rates by 36 months.

Results: With a data cut-off date of 07 Dec 2016, a total of 108 patients were evaluated; 28 patients in NIL group (group 1), 28 patients in high-dose IM group (group 2), and 52 patients in standard-dose IM group (group 3). Median follow-up duration from enrollment was 36 months (range, 1-36), 45 months (range, 12-85), 45 months (range, 12-76), respectively for each group, respectively. All patients in group 1 remained NIL treatment, 18 patients in group 2 crossed over to NIL 400mg QD due to intolerance (n=4) and lack of response (n=14), in group 3, 23 patients switched to other treatment due to intolerance (n=7), lack of response (n=8), failure (n=1), or treatment change (n=5). Among all treated patients (13.2%) but also in imatinib (2.7%) and nilotinib (10%), including patients without any symptoms. This indicates possible PH onset among patients treated with imatinib or nilotinib, as well as with dasatinib. Although TRGP values obtained by echocardiography might not be fully compatible with those by cardiac catheterization, the results suggested that noninvasive echocardiography is sensitive for screening PH and is also effective for easily screening groups of patients with suspect subclinical PH among patients treated with any available TKIs. Careful screening with echocardiography is necessary especially for older patients who have received TKIs for a long time.

Summary/Conclusions: PH is a rare but life-threatening adverse event for dasatinib-treated patients, and its definitive diagnosis is made by cardiac catheterization. However, cardiac catheterization is too invasive for PH screening of the many patients with TKIs who do not have any symptoms. Our study, by using echocardiography, detected TRGP elevation not only in dasatinib treated patients (13.2%) but also in imatinib (2.7%) and nilotinib (10%), including patients without any symptoms. This indicates possible PH onset among patients treated with imatinib or nilotinib, as well as with dasatinib. Although TRGP values obtained by echocardiography might not be fully compatible with those by cardiac catheterization, the results suggested that noninvasive echocardiography is sensitive for screening PH and is also effective for easily screening groups of patients with suspect subclinical PH among patients treated with any available TKIs. Careful screening with echocardiography is necessary especially for older patients who have received TKIs for a long time.

PB1816 COMPARATIVE ANALYSIS OF PULMONARY HYPERTENSION IN THE 105 CML PATIENTS TREATED WITH IMATINIB, NILOTINIB AND DASATINIB
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Background: Pulmonary hypertension (PH) has been reported as a serious adverse event in chronic myeloid leukemia (CML) patients treated by dasatinib. French group reported incidence of PH diagnosed by cardiac catheterization as 0.45% (13 of 2,900 patients) in symptomatic patients treated with dasatinib. Dasatinib-related PH usually resolves after cessation of treatment, but it can be fatal, as two deaths in France and one in Japan have been documented.

Aims: To clarify the incidence of tyrosine kinase inhibitor (TKI)-related PH, we noninvasively screened CML patients who have been given imatinib, nilotinib or dasatinib by echocardiography.

Methods: 105 patients with CML in chronic phase (CP) who received TKI were enrolled in this study between 2014 and 2015. Nine patients with newly diagnosed CML in CP prior to TKI treatment were added as control. Patients underwent echocardiography to evaluate TRP values of tricuspid regurgitation pressure gradient (TRPG), which relates to severity of PH. Patients with TRPG values >31mmHg were suspected of PH onset according to European Society of Cardiology criteria. All patients gave informed consent.

Results: Patients were divided into 3 groups by the TKIs they used at the time of study enrollment; 37 patients on imatinib, 30 on nilotinib and 38 on dasatinib (Table 1). In imatinib group, patients’ age was significantly higher, and duration of treatment was also longer than those of the 2nd generation TKIs. Echocardiography revealed mean values of TRPG as 22.7, 23.1 and 23.4mmHg in imatinib, nilotinib and dasatinib groups, respectively (p=0.087), and these values were higher than that in the newly diagnosed CML patients (19.0mmHg), though without significance (p=0.38). None of the 105 patients (8.6%) presented with an elevated TRPG>31mmHg, suggesting the presence of PH; 1 of 37 (2.7%) in imatinib group, 3 of 30 (10.0%) in nilotinib group, and 5 of 38 (13.2%) in dasatinib group. Three patients complained of dyspnea, while the remaining 6 were asymptomatic. We found no apparent risk factors associated with TRPG elevation, however, there were trends toward correlation of age and TRPG values in nilotinib and dasatinib treated patients, and treatment duration and TRPG values in nilotinib treated patients. Imatinib dosage tended to inversely correlate with TRPG value, suggesting that imatinib might decrease pulmonary arterial blood pressure in a dose-dependent manner.

Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Patients</th>
<th>Age (years)</th>
<th>Duration of Treatment (months)</th>
<th>TRPG (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imatinib</td>
<td>37</td>
<td>57.6 ± 10</td>
<td>105 ± 39</td>
<td>22.7 ± 2.4</td>
</tr>
<tr>
<td>Nilotinib</td>
<td>30</td>
<td>57.9 ± 10</td>
<td>105 ± 39</td>
<td>23.1 ± 2.6</td>
</tr>
<tr>
<td>Dasatinib</td>
<td>38</td>
<td>58.1 ± 10</td>
<td>105 ± 39</td>
<td>23.4 ± 2.7</td>
</tr>
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</table>

Summary/Conclusions: In this study, we investigated the incidence of PH in CML patients treated with TKIs. Noninvasive echocardiography was performed in 105 CML patients treated with imatinib, nilotinib or dasatinib. There were no cases with PH onset according to European Society of Cardiology criteria. There were trends toward correlation of age and TRPG values in nilotinib and dasatinib treated patients, and treatment duration and TRPG values in nilotinib treated patients. Imatinib dosage tended to inversely correlate with TRPG value, suggesting that imatinib might decrease pulmonary arterial blood pressure in a dose-dependent manner.
TKI resistance. Here we present our data concerning prognostic significance of BCR-ABL1 kinase domain mutations dynamics in Russian CML patients according the follow-up study having been performed during the last 10 years.

Aims: To determine the frequency dynamics of BCR-ABL1 mutations in CML patients and its prognostic significance.

Methods: In this study we have included 1077 TKI resistant CML patients from 112 hospitals in 77 Russian cities having been observed during the period from 2006 to 2016. BCR-ABL1 kinase domain point mutations in mRNA samples from peripheral blood cells were analyzed by means of PCR followed by Sanger sequencing. Statistical analysis was performed using SPSS 22.0 (IBM, USA) and Excel 2013 (Microsoft, USA). Critical p-value was set to 0.05.

Results: 1077 TKI resistant CML patients were analyzed, among them were 41,5% men (n=447) and 58,5% women (n=630), median age – 50 (from 15 to 74). BCR-ABL1 mutations were found in 30,8% (332/1077) CML pts. We have detected a total of 415 mutations in 332 patients, giving rise for 58 different mutation variants. Mutation associated resistance rate was higher in women to compared to men (44,2% vs 34,1%; p=0.028). The lowest resistance rate was associated with e13a2 transcript. No statistically significant differences in frequency spectrum of known BCR-ABL1 mutations were observed between men and women in both phases of TKI treatment. The median follow-up was 60 months (range 24-82 months). The response rates and the survival probabilities were higher in patients with e13a2 transcript compared to patients with e14a2 transcript (N=174), but the differences were not significant: MR by 12 months, 66% vs 72%, p=0.244; MR4.5 by 36 months, 56% vs 66%, p=0.067; estimated cumulative incidence of MMR, 82% vs 88%, p=0,135; estimated cumulative incidence of MR4.5, 60% vs 69%, p=0,101; estimated EFS, 88% vs 93%, p=0.547; estimated EFS-CR 89% vs 94%, p=0.2. The responses and the survival probabilities of patients co-expressing the e13a2 and the e14a2 transcripts (N=30) were similar to or even better than the ones of e14a2 patients. Grouping together the patients with e14a2 transcript alone and the patients with co-expression of both transcripts (N=174+30=204), and comparing them to patients with e13a2 transcript alone (N=174), no statistically significant differences in response rates were observed: estimated PFS of 89% vs 90%, p=0.505 and estimated OS, 91% vs 92%, p=0.336, respectively.

Summary/Conclusions: As far as different BCR-ABL1 kinase domain mutations are associated with various types of mutation associated resistance to TKI treatment, the detection of trends in mutation distribution in CML patients receiving TKI treatment is very important for long time treatment strategy decision making, and is of high importance of resistance. We believe these data highlight the regional difference of mutation profiles should also be considered. Therefore, to enable correct triggering of particular types of TKI for CML treatment it is necessary to obtain data of when, which and where a particular type of BCR-ABL1 mutation is prone to appear in a distinguished cohort of CML pts.

PB1819

IMPACT OF BCR-ABL1 TRANSCRIPT TYPE IN CHRONIC MYELOID LEUKEMIA TREATED FRONTLINE WITH NILOTINIB


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Background: Imatinib (IM) monotherapy remains an acceptable option to treat newly diagnosed CML patients with chronic myeloid leukemia (CML) in the chronic phase (CP). Hydroxyurea (HU) is effective in controlling elevated white blood cell counts and has been widely used to treat CML prior to the era of tyrosine kinase inhibitors (TKIs). The combinations of IM and HU have been tested in vitro and showed a additive suppression of CML CFU-GM cells. Combinations of IM and hydroxyurea (HU) or cytarabine have shown promise in vivo, but no data are available for the combination of IM and HU in CML.

Aims: The East German Study Group conducted a phase I study to identify the dose of HU in combination with standard dose IM (400mg daily) that would result in mild myelosuppression (white blood cell count 3,000-4,000/ml). Start- ing dose IM was 150mg daily, and HU was increased if toxicities were serious enough to reach a maximum of 3,000 mg daily. According to protocol, 500mg HU was identified as the starting dose for the randomized phase II study which tested the combination vs standard dose IM, with the rate of major molecular response (MRM) at 18 months as the primary endpoint.

Methods: Two hundred and twenty patients with newly diagnosed CP-CML were included in the phase I study. Additional 93 patients were enrolled in the phase II of the study, 5 of whom were excluded. With ratio 2:1 in phase II, 88 patients were randomized to the IM/HU (n=59) and IM (n=29) arm, respectively.
Three patients (2 IM/HU, 1 IM) were lost to follow-up. As prospectively designed, all available IM/HU patients (n=77) were accounted for. According to the study protocol, patients from the CML IV study were to be added to obtain equal numbers for analysis. To arrive at a total of 77 IM patients, from study IV another 49 patients were selected by propensity score matching. The median age of the 154 patients was 55 years (range 18 – 82). The ELTS prognostic score was available for 141 patients and was high in 8 (5.7%), intermediate in 35 (24.8%), and low in 98 (69.5%), with no significant differences between treatment groups.

**Results:** The 5-year overall survival (OS) / progression-free survival (PFS) probabilities were 90.4 and 86.7% in the IM/HU and twice 84.9% in the IM arm respectively. IM/HU-mediated probability of complete cytogenetic response (CCR) at 6, 12, and 18 months were 54.3, 84.0, and 93.7%. In the IM arm, the corresponding numbers were 70.4, 84.9, and 83.3% (p<0.05 significant). Primary endpoint was MMR rate at 18 months. There was no significant difference between IM/HU (65.8%) and IM (66.0%). At 6 months, MMR status (IM/HU) vs 41.1% (IM/HU) and at 12 months 41.9 (IM/HU) vs 58.9% (not significant). Time to event analyses of OS and PFS did not result in significant differences; neither did group comparisons between the probabilities of CCR and MMR. The median HU dose was 500mg (range 152-3000); the median IM dose was 400 mg (range 145-617mg). The gross number of adverse events in general or of adverse events of grade 4 were not different between the two arms, but cumulative incidences showed an earlier occurrence in the IM/HU than in the IM arm (p=0.0343, Gray test).

**Summary/Conclusions:** Compared to Imatinib only, the combination of Imatini and HU resulted in a lower MMR rate at 6 months but a similar MMR rate at 18 months. Furthermore, IM/HU was associated with more early adverse events. There was no indication of a beneficial effect in the treatment of CML patients in 1st chronic phase using the combination of IM with HU.

PB1820

**A MULTICENTER, OBSERVATIONAL, AMBISPECTIVE STUDY EVALUATING EFFICACY AND SAFETY OF GENERIC IMATINIB COMPARED TO GLEEVEC IN CHRONIC MYELOGENOUS LEUKEMIA IN CHRONIC PHASE - 3 MONTHS RESPONSE ANALYSIS**

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**Background:** The efficacy of branded imatinib (Gleevec) in the first-line treatment of chronic myeloid leukemia (CML) has been demonstrated in several clinical studies. However, there are few consistent data in the literature on the efficacy and adverse effects of generic formulations of imatinib. In Brazil, CML patients have been treated in the national public health system with generic imatinib since June 2013.

**Aims:** The present study aims to evaluate the efficacy and safety of generic imatinib in the treatment of CML in comparison with the reference drug (Gleevec) in the first three months of imatinib treatment.

**Methods:** This is a multicenter, observational, ambispective, cohort-type study. The study was initiated in January 2015 with the intended participation of 17 Brazilian centers. In the prospective group, were selected chronic phase CML patients who started their first-line treatment with generic imatinib between January 2015 and October 2016, whereas retrospective group was treated with Gleevec between January 2010 and December 2011. All patients started imatinib less than six months from diagnosis. Study data were collected and managed using REDCap electronic data capture tools. Demographic data were collected at diagnosis: age, gender, Sokal, Hasford, EUTOS score, comorbidities, cytogenetics, BCR-ABL transcript type. The definition of the responses followed the five-point criteria of the European Leukemia Net 2013. Adverse events were assessed based on the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0.3, 2010. Statistical analysis: SPSS version 21.0 was used applying the chi-square and t-test, when adequate. All analysis considered p-value <0.05 as significant.

**Results:** Ten centers were recruited 177 patients in the retrospective group and 68 patients in the prospective group so far. For this preliminary analysis, response data from 132 patients were available (47 from prospective and 85 from the retrospective groups). The median age of patients was 54 years in the prospective group and 46 years in the retrospective group (P=0.12). Sokal scores were prospectively and retrospectively, respectively: low risk 42%/52%; intermediate risk 42%/31%; high risk 45%/67% (P=0.48). There was no difference between the groups concerning gender, Hasford, EUTOS scores, ECOG, blood cell counts at diagnosis and before starting imatinib and BCR-ABL transcripts. Regarding responses, there was no difference in the hematological complete cytogenetic responses and rate of BCR-ABL transcripts >10% at three months. However, there was a higher rate of failure at three months according to the ELN 2013 criteria in the prospective group (14.9% versus 4.7% Gleevec group, P=0.04). There was no significant difference in grade 3 and 4 hematological and non-hematological toxicity, but there was one early death in the prospective group (acute peripheral arterial occlusion and renal failure). Four patients discontinued imatinib: one from Gleevec group (resistance) and three from the generic group due to intolerance (1) and resistance (2).

**Summary/Conclusions:** According to ELN-2013 criteria, there was a higher rate of failure at three months, but no difference in toxicity. The register is ongoing; the confirmation of this data and the impact in prognosis will be evaluated in the long-term follow-up, after increasing the number of patients.

PB1821

**COMPLEX ADDITIONAL CHROMOSOME ABERRATIONS IN PH-POSITIVE CELLS IMPACT ON CHRONIC MYELOID LEUKEMIA PATIENTS’ SURVIVAL IN THE ERA OF TYROSINE KINASE INHIBITORS**

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**Background:** Additional chromosomal aberrations (ACA) as marker of clonal evolution in chronic myeloid leukemia (CML) patients were previously noted in association with resistance to therapy. The presents of ACA have been associated with a worse prognosis for survival in the pre-TKI era. The ACA classification proposed earlier was based only on its frequencies. Whereas ACA's clinical impact had not yet been clearly established.

**Aims:** The aim of our study was to evaluate the long-term impact of the ACA presence in Ph-positive cells in CML patients on TKI treatment results.

**Methods:** 30 patients with ACA in Ph-positive cells treated in our center from 2005 to 2015 years were included in this study. Cytogenetic analyses of at least 20 Giemsa-banded bone marrow metaphases were interpreted per ISCN 2013. We analyzed overall survival (OS) and cumulative incidence of CML-related death on TKI treatment. Cox regression was used for multivariate survival analysis, that included next covariates: number of ACA, type of ACA, age, TKI type, CP or AP at diagnosis. OS was estimated by Kaplan-Meier method with log-rank test for comparison. Cumulative incidence of CML-related death was estimated into consideration the presents of competing risks (CML-unrelated death) using Gray’s test for comparison between groups.

**Results:** Median follow-up period in ACA group (n=30) was 51 months (3-124). ACA at diagnosis were detected in 16 (53%) of 30 patients. Chronic phase patients at diagnosis were detected in 23 (77%) patients. At 3 months 18 patients were in CP, 2 patients were in AP; 4 patients from this group had «major-route» ACA. Accelerated phase was defined in 7 (23%) patients. In that group treatment of 6 patients was started with Imatinib and Dasatinib was given initially for one patient. «Major-route» ACAs (trisomy 8, 16p, 22q(20);q22;q34;q11, t(17;11), t(15;19) ) were detected in 16 (53%) of 30 patients. Complex aberrations (2 ACA and more) were revealed in 7 (23%) patients, 4 patients from this group had «major-route» ACA. 10-years OS in the whole ACA group was 67%, 10-years cumulative incidence of CML-related death was 23%. Number of ACA (p=0.03, HR=13.2) and age (p=0.03, HR=1.14) had statistical significance influence on survival by regression analysis. 10-years OS was 31% and 77% (p<0.05) in patients with complex ACA and single ACA respectively. 10-years cumulative incidence of CML-related death was 54% for patients with complex aberrations versus 10% for single ACA patients (p<0.05) (Figure 1).

**Summary/Conclusions:** Our results showed that TKI treated CML patients with complex ACAs have a higher risk of progression and death in comparison with single-ACA patients.

Figure 1.
Response was analysed, as well as rate of treatment failure at 6 and 12 months. European LeukemiaNet (ELN) 2013 guidelines, rate of optimum therapeutic with TKIs in our institution during period from August 2012 to February 2017. Cohort of 101 adult patients with CP-CML was analysed, treated Methods: imatinib (GI), although there is a growing number of countries in which it is there is still limited data and some concerns about the effectiveness of generic.... I. Dmytrenko1,*, V. Fedorenko1, Z. Martina2, V. Sholoyko2, T. Shlyakhtychenko1, Z. Minchenko1, I. Dyagii2 1Immunogenetic Laboratory, 2Hematology and Transplantology Department, National Research Center for Radiation Medicine, Kyiv, Ukraine Background: Several types of transcripts can be produced during chromoso- translocation, which lead to formation of the BCR/ABL fusion gene in patients with chronic myeloid leukemia (CML). Previous results of a few large studies risk score and the proportion of patients with additional chromosomal abnormalities in Ph-positive cells. No correlation of transcript type with age or sex was observed. Transcript e13a2 was associated with higher WBC (120x10^9/L vs 95.3x10^9/L, p=0.02) and lower baseline percentage of eosinophils (p=0.041). No differences were found in other differential counts of peripheral blood. Comparison of patients treated with nilotinib was 44 months (range 1-137). A qualitative RT-PCR for BCR/ABL1 transcript was performed at diagnosis. The patients who achieved CCR but did not have major molecular response (MMR) as well as patients with rare BCR/ABL1 transcripts and coexpression were excluded from the analysis. Probability of overall survival (OS), progression-free survival (PFS) and event-free survival (EFS) was calculated using Kaplan-Meier method. Event in EFS was defined as death of a patient on treatment for any reason, progression of disease, or loss of CCR or MMR. Differences between groups were assessed using log-rank, χ^2-tests and Mann-Whitney U-tests. Cumulative probability of CCR, MMR, MR4.0 (BCR/ABL<0.01%) and loss of CCR and MMR was assessed using Kaplan-Meier method. Results: The median follow up was 23 (range 4 – 82) months. The groups with both of the BCR/ABL1 main transcripts were comparable for the disease phase. Sokal risk score and the proportion of patients with additional chromosomal abnormalities in patients who were newly diagnosed with CML-CP enrolled into ENESTchina trial. Responses based on molecular and cytogenetic outcomes were measured according to the European LeukemiaNet recommendations, and patient-reported HRQoL profile was measured by the SF-36 health survey. Results: Of 143 patients, 132 were eligible for analysis. The first group consisted of 53 patients treated with GI (Anzovin). According to European LeukemiaNet (ELN) 2013 guidelines, rate of optimum therapeutic response was analysed, as well as rate of treatment failure at 6 and 12 months. The second group consisted of 48 patients switched from OI to GI, in which the rate of achieved therapeutic response at the time of switching and the rate of maintenance of CCyR, MMR and MR4 after a minimum of 12 months under therapy with GI were both analysed. In order to investigate safety of GI, in both groups rate of hematological and non-hematological adverse effects (AEs), all grades according CTC AE criteria, were analysed. Results: Analysis of the response by ELN criteria in the group with GI showed that at 6 months 33/53 (62.3%) patients achieved CCyR, BCR-ABL<1% was in 27/52 (51.9%) patients, while 15/52 (28.8%) of patients achieved MMR. At 12 months of therapy, 35/49 (71.4%) of analysed patients achieved CCyR, and 25/49 (48.9%) achieved MMR. ELN criteria for treatment failure at 6 months were 12/53 (22.6%) patients, while at 12 months ELN criteria satisfied 13/49 (26.5%) of analysed patients. After 18 months of therapy with GI the rate of CCyR was 35/46 (76.1%) and MMR was 28/45 (62.1%) and showed trend of increase. During the median follow-up period of 23.8 months 3 patients have progressed to blast phase and total of 7 patients died. In the second group, in time of switching from OI to GI, the rates of achieved CCyR, MMR and MR4 were 82.5%, 65.8% and 49% of patients respectively. The rate of maintenance of previously achieved CCyR was 95%, of MMR 88% and of MR4 72% in the course of the median duration of GI exposure of 37.8 months. When comparing first and second group respectively, the rates of patients which have been switched to 2nd generation of TKI because of the failure or intolerance to imatinib were 27.8% vs. 24.8%, and 60.5% vs. 64.5% of them achieved secondary optimal therapeutic response (CCyR plus MMR), while 25% vs. 20% of them have been sent to BMT. Group switched from OI to GI had not significantly different compared to original imatinib (p=0.24, p=0.991). Furthermore, the rate of grade 3-4 hematological AEs were similar in both groups (13% vs 15%, p=0.952).

Summary/Conclusions: Results of this study with extended follow-up of more than four years are further evidence that the use of generic imatinib is at least non-inferior to the original imatinib regarding efficacy both when used initially or as a subsequent regimen for branded imatinib.

PB1824 ACHIEVING OPTIMAL RESPONSE AT 12 MONTHS IS ASSOCIATED WITH A BETTER HEALTH-RELATED QUALITY OF LIFE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA: A PROSPECTIVE, LONGITUDINAL, SINGLE CENTER STUDY Q. Jiang1,*, H. Wang2, L. Yu1, D. Milijkovic3, X. Huang1 1Peking University People’s Hospital, Peking University Institute of Hematology, 2Peking University Clinical Research Institute, Beijing, China, 3Novartis Pharma AG, Basel, Switzerland Background: Health-related quality-of-life (HRQoL) profile is now recognized as an important component in the management of Chronic myeloid leukemia (CML). Aims: To explore the HRQoL profiles of patients with CML in the chronic phase (CP) who were treated with front-line imatinib or nilotinib, in order to assess the relationship between early response and HRQoL outcomes. Methods: A prospective, longitudinal, single center study was conducted to assess the response to treatment with imatinib or nilotinib and the HRQoL profile of patients who were newly diagnosed with CML-CP and enrolled into ENESTchina Trial. HRQoL profiles based on SF-36 were measured according to the European LeukemiaNet recommendations, and patient-reported HRQoL profile was measured by the SF-36 health survey. Results: Fifty-nine patients were randomly assigned to receive imatinib (n=31) or nilotinib (n=28). In multivariate analysis, the use of nilotinib was identified as an independent factor affecting the achievement of optimal response at 6 months (OR=3.9, 95% CI, 1.0-14.9; P =0.043) and 12 months (OR=5.6, 95% CI, 1.7-17.9; P =0.004). With a median follow-up of 60 months, the probabilities of failure-free survival (all P values <0.001) and progression-free survival (all P Values <0.05) at 5 years were significantly higher in patients who achieved optimal response at 3, 6, or 12 months than those who achieved non-optimal response (warning or failure), and overall survival rate at 5 years was significantly higher in those who achieved optimal response at 12 months (P =0.047). Achieving optimal response at 12 months was associated with better role limit- ations due to physical health problems (P =0.0019) and role limitations due to emotional problems (P =0.0110) and was the sole factor associated with significantly improving physical component summary over time (P =0.0160). In addition, achieving optimal response at 6 months had a tendency of high physical functioning (P =0.0674), social functioning (P =0.0571), and role limitations due to emotional problems (P =0.036) and improved physical component summary over time (P =0.0160). Background: Tyrosine kinase inhibitors (TKIs) are the golden standard in the treatment of chronic phase chronic myeloid leukemia (CP-CML) due to their high efficacy and mild toxicity profile. Because of the high price of these drugs, the use of generics is encouraged to reduce health care costs. In the literature, there is still limited data and some concerns about the effectiveness of generic imatinib (GI), although there is a growing number of countries in which it is used instead of first-line imatinib (OI). Aims: The objective of this study was to evaluate efficacy and safety of GI in newly diagnosed CP-CML patients treated with front-line GI and in patients switched from OI to GI. Methods: Cohort of 101 adult patients with CP-CML was analysed, treated with GI, and followed up during period from August 2012 to February 2017. First group consisted of 53 patients treated with GI (Anzovin). According to European LeukemiaNet (ELN) 2013 guidelines, rate of optimum therapeutic response was analysed, as well as rate of treatment failure at 6 and 12 months.

PB1823 ANALYSIS OF GENERIC IMATINIB EFFICACY IN CHRONIC MYELOID LEUKEMIA TREATMENT: MORE THAN FOUR YEARS OF EXPERIENCE IN SOUTHERN SERBIA I. Cojbasic1, L. Macukanovic Golubovic1,2, M. Vucic1,2, I. Tijanic1,2 1Medical Faculty, University of Nis, 2Clinic of Hematology and Clinical Immunology, Clinical Center Nis, Serbia, Nis, Serbia Background: Tyrosine kinase inhibitors (TKIs) are the golden standard in the treatment of chronic phase chronic myeloid leukemia (CP-CML) due to their high efficacy and mild toxicity profile. Because of the high price of these drugs, the use of generics is encouraged to reduce health care costs. In the literature, there is still limited data and some concerns about the effectiveness of generic imatinib (GI), although there is a growing number of countries in which it is used instead of first-line imatinib (OI). Aims: The objective of this study was to evaluate efficacy and safety of GI in newly diagnosed CP-CML patients treated with front-line GI and in patients switched from OI to GI. Methods: Cohort of 101 adult patients with CP-CML was analysed, treated with GI, and followed up during period from August 2012 to February 2017. First group consisted of 53 patients treated with GI (Anzovin). According to European LeukemiaNet (ELN) 2013 guidelines, rate of optimum therapeutic response was analysed, as well as rate of treatment failure at 6 and 12 months.

PB1822 BCR/ABL1 TRANSCRIPT E13A2 IS ASSOCIATED WITH HIGHER CUMULATIVE PROBABILITY OF LOSS OF MAJOR MOLECULAR RESPONSE IN CML PATIENTS TREATED WITH NILOTINIB AS THE 2-ND LINE THERAPY I. Dmytrenko1,*, V. Fedorenko1, Z. Martina2, V. Sholoyko2, T. Shlyakhtychenko1, Z. Minchenko1, I. Dyagii2 1Immunogenetic Laboratory, 2Hematology and Transplantology Department, National Research Center for Radiation Medicine, Kyiv, Ukraine
SECOND-LINE TYROSINE KINASE INHIBITORS IN CHRONIC PHASE - CHRONIC MYELOGENOUS LEUKEMIA (CML-CP)

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Background: Achieving deep molecular response, ≥4.5-log reduction (MR4.5; BCR-ABL1 on the International Scale [IS] ≤0.0032%), is one of the important prerequisites for attempting treatment-free remission. Limited information is available on comparative rates of MR4.5 between nilotinib and dasatinib in second-line (2L).

Aims: This study aims to investigate time to achieving MR4.5 and major molecular response (MRM; ≥3-log reduction or ≤0.1% in BCR-ABL1 on IS) in CML-CP patients (pts) treated with nilotinib vs dasatinib in 2L.

Methods: An online physician panel approach was used to recruit oncologists (N=141) globally to conduct a retrospective medical chart audit. Physicians were instructed to select up to 4 pts who met the following criteria via a random letter generation scheme for the first letter of pt’s last name: diagnosed with CML-CP at age ≥18 years, initiated 2L nilotinib or dasatinib between 1/1/11 and 3/31/12, and were followed-up for at least 6 mos post-initiation of 1L TKI. Multivariate Cox proportional hazards models accounting for country clustering random effects were used to assess the effect of nilotinib vs dasatinib on time to MR4.5 and MMR, adjusting for age, gender, Sokal risk score at diagnosis, hydroxyurea use before 1L TKI, 1st vs 2nd generation TKI as 1L, and reasons for 1L TKI discontinuation. Adjusted hazard ratios (HR) and 95% confidence intervals (CIs) were reported. Adverse events (AEs) were also described.

Results: The study included 236 pts from Australia, Brazil, France, Germany, Italy, and Netherlands treated with nilotinib (N=115[49%]) or dasatinib (N=121[51%]) in 2L. Both groups had a similar mean follow-up of 23 mos, mean age of 57 yrs, and were 35% female. 8% of 2L nilotinib and 22% of 2L dasatinib pts were instructed to select up to 4 pts who met the following criteria via a random letter generation scheme for the first letter of pt’s last name: diagnosed with CML-CP at age ≥18 years, initiated 2L nilotinib or dasatinib between 1/1/11 and 3/31/12, and were followed-up for at least 6 mos post-initiation of 1L TKI. Multivariate Cox proportional hazards models accounting for country clustering random effects were used to assess the effect of nilotinib vs dasatinib on time to MR4.5 and MMR, adjusting for age, gender, Sokal risk score at diagnosis, hydroxyurea use before 1L TKI, 1st vs 2nd generation TKI as 1L, and reasons for 1L TKI discontinuation. Adjusted hazard ratios (HR) and 95% confidence intervals (CIs) were reported. Adverse events (AEs) were also described.

Summary/Conclusions: The major finding of this study is that ANFIS models using the morphometric parameters, available at diagnosis of chronic phase of the CML, may improve prediction of CCgR at 6, 12 and 18 months on imatinib therapy, in comparison to the EUTOS score being the standard prognostic scoring system and regression models using the same inputs. Using neuro-fuzzy computationally intelligent ANFIS models with morphometric parameters in conjunction with EUTOS score improves prediction of CCgR. Validation on larger groups of patients is needed, but these findings indicate that neuro fuzzy models could aid in individual CML patient risk stratification.

PB1827

A NATIONWIDE OBSERVATIONAL STUDY OF PONATINIB IN CHRONIC MYELOGENOUS LEUKEMIA OUTSIDE CLINICAL TRIALS

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Background: In December 2014 the oral tyrosine kinase inhibitor (TKI), ponatinib was granted an accelerated approval by the FDA based on promising results from the phase II PACE (Ponatinib Ph-ALL and CML evaluation) trial. Yet, nowadays the use of this drug is limited because of safety issues, most notably increased risk of vascular complications. Currently, there is very little real-life information regarding the use of ponatinib outside clinical trials.

Aims: The purpose of the current study is to characterize patients who received ponatinib and to assess the safety profile and efficacy of ponatinib outside clinical trials.

Methods: Data from electronic charts of chronic myeloid leukemia (CML) patients treated with ponatinib were analyzed.

Results: Patients characteristics: Between 4.2011 and 1.2017 (69 months) 37 patients with an initial diagnosis of CML in 9 medical centers in Israel received ponatinib. The median age at time of treatment was 43 years (range: 9 to 82) and approximately half of the patients had chronic phase CML (N= 19, 53%). Based on their medical history, 36% (N=12) were at increased risk for vascular complications. Pre-ponatinib treatments: Patients received at least one other TKI and most received at least two different TKI-
based regimens (N=28, 76%). Nine patients (25%) underwent hematopoietic stem cell transplantation (HSCT) prior to ponatinib. The time that lapsed from diagnosis until ponatinib initiation ranged considerably (from 1 to 215 months, median 47 months). Indications for ponatinib switch: 26% of patients (N=9) switched to ponatinib because T315I mutation was detected. The remaining switched either because of progressive disease, i.e. accelerated (N=5, 14%) or blast crisis (17%, N=8, 17%) phases, and 14 (39%) because they experienced loss of previous molecular or cytogenetics response. Only 5% (N=2) switched because of unacceptable side effects to previous treatments. Treatment with Ponatinib: Patients received ponatinib for a median time of 14 months (range: 1 to 51). The drug started at the recommended dose of 45 mg/day only in 60% (N=22) of patients and in 24% of them (N=9) the dose was reduced during treatment. The median survival time of patients with ponatinib was 38 months (95% CI: 30 to 47 months) (Figure 1). Patients died because of cerebrovascular event (N=1), sepsis (N=2) or graft vs host disease that developed shortly after HSCT (N=1). Response assessment: Response assessment as available for 32 patients (86%). Seventy percent (N=22) achieved molecular response, of which 60% (N=13) achieved at least major molecular response. The median time to maximal response was 7 months (range: 3 to 28 months). Drug discontinuation: Twenty four percent (N=9) discontinued ponatinib after a median of 7 months (range: 1 to 18 months) because of disease progression (N=6) or severe adverse effects in two patients (cerebrovascular event and severe pancytopenia).

Figure 1.

Summary/Conclusions: In our cohort ponatinib was almost always used in patients who experienced treatment failure to previous TKIs. Still, molecular response was achieved in most patients, even in those with progressive disease in accelerated or blastic phases. The vast majority of patients received reduced doses of ponatinib and although more than one third of patients were at-risk for vascular events, only two patients developed serious life-threatening vascular episodes. In heavily pre-treated patients, ponatinib is effective and safe and can be considered even in patients with cardiovascular risk factors.

PB1828
MOLECULAR RESPONSE TO THERAPY WITH TYROSINE KINASE INHIBITORS IN PATIENTS WITH BCR-ABL1(+): CHRONIC MYELOID LEUKEMIA PRESENTING WITH AN ISOLATED THROMBOCYTOSIS AT THE ONSET
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Background: Generally, chronic myeloid leukemia (CML) and essential thrombocythemia (ET) are characterized by distinct clinical and laboratory characteristics, including the spectrum of genetic abnormalities - Philadelphia chromosome (Ph) and BCR-ABL1 fusion transcripts in CML and JAK2, CALR or MPL gene mutations in ET. Therefore, even in the presence of overlapping features in some cases, the correct diagnosis can be assigned. However, in rare cases Ph chromosome and BCR-ABL1 fusion transcripts can be found in otherwise typical ET. The number of reported cases of subsequent course of the disease and the response to tyrosine kinase inhibitors (TKI) in such patients with BCR-ABL1-positive thrombocytosis is largely unknown.

Aims: To report the clinical course and response to TKI in patients (pts) with CML presenting with isolated thrombocytosis at the onset.

Methods: In total, 31 pts with Ph(+) and/or BCR-ABL1 positive isolated thrombocytosis and a moderate or absent leukocytosis were retrieved from the hospital database. The cohort comprised 17 females and 14 males, at a median age of 47 years (range 23-86). Diagnosis was based on blood and bone marrow morphology and differential, cytogenticors and/or molecular testing according to the WHO criteria (2008). Molecular monitoring was carried out using Xpert BCR-ABL Monitor or Xpert BCR-ABL Ultra tests (Cepheid). In total, follow up data for at least 6 months (mean 65 months) are available for 25 patients treated with TKI as a first-line therapy.

Results: At diagnosis the median leukocyte count was 22 x109/L (range 6-36) and platelet count – 316 x109/L (range 770-2815). Splenomegaly was found in 5 pts (16%). Only one patient was diagnosed in accelerated phase as the remaining presented in chronic phase at diagnosis. Interestingly, 4 pts (12.9%) had a history of an antecedent solid tumor. All patients enrolled in the study were BCR-ABL1(+): b3a2 (n=16) or b2a2 (n=15). Karyotypes were available in 21 patients and classified in 16 of them (89%), with 12 patients (71%) had a cryptic translocation was detected as well as a variant Ph in the remaining 2 pts (8.7%). Imatinib was used as a first line therapy in 15 pts and optimal response was achieved in 53.3% (8/15), while 5 were switched to a second line, and 2 - to a third line therapy. First-line treatment with nilotinib in 10 patients resulted in optimal response in 80% (n=8). In patients with the best molecular response (MR) was achieved in 80% (n=20), including deep MR in 56% (14/20). One pt was lost of follow up after optimal response was registered. No response was documented in 4 pts (16%) and progression to blast crisis developed in 2 of them. The mean OS was estimated 143 months and the cumulative propor-
tional surviving at 5 years was 91%.

Summary/Conclusions: Interestingly, CML presenting with isolated thrombocytosis at diagnosis in our cohort had high proportion of antecedent malignancies and high incidence of cryptic Ph translocation without any specific correlation with the transcript types. However, the clinical course and molecular response to TKI therapy was similar to the reported in CML in general. Acknowledgements: Partial support by the National Science Fund.
PB1830
SHOULD SWITCHING TO SECOND GENERATION TKIS BE A RULE IN PATIENTS WITH CP-CML AFTER 3-6 MONTHS OF IMATINIB TREATMENT? RETROSPECTIVE ANALYSIS OF CML PATIENTS TREATED IN A SINGLE BRAZILIAN CANCER CENTER

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Background: Early molecular response is an important predictor for survival and therapy-free remission in chronic myeloid leukemia (CML). The current guidelines define BCR-ABL1 <10% at 3 months and/or 1-10% at 6 months as warning signals; however, it is not clear if switching imatinib to second generation TKIs in this scenario improves responses and overall survival in patients outside clinical trials.

Aims: To analyze the proportion of patients with major molecular response (MMR) at 12 months according to the molecular response at 3 and 6 months in a cohort of CML population, not enrolled in clinical trials and treated only with imatinib. Also evaluate the incidence of molecular responses log3.0, log4.0 and log4.5 at any time in patients who did not switch to second generation TKIs.

Methods: Retrospective analysis of all 226 patients diagnosed with CML from January 2007 until January 2015 in our hospital. The exclusions criteria were: advanced phases, inclusion in clinical trial, treatment with second-generation TKI in the first 12 months (due to toxicity or failure). The molecular response was evaluated according ELN recommendations: RQ-PCR assessment of BCR-ABL1 levels every 3 months until achievement of MMR, with molecular evaluation every 3-6 months afterward. All samples were analyzed in the same laboratory which was standardized since 2007.

Results: In the first cohort, 150 patients with CML chronic phase were analyzed. Optimal molecular responses by the ELN at 3 and 6 months were predictors of MMR by 12 months (94% vs 6%, p<0.0001 at 3m, 89.3% vs 10.7%, p<0.0001 at 6m), but there was no overall survival benefit. A second cohort with 119 patients received only imatinib, with a medium follow-up time of 71 months (13-117m), MMR was achieved by 80% of this imatinib-only group after 12 months and by more than 90% after 36 months (Figure 1). Patients with BCR-ABL1 <10% at 3 months and/or <1% had a higher probability of achieving MMR3, MMR4 and MMR4.5 at any time.

Figure 1.

Summary/Conclusions: Our study shows that around 30% of the patients that do not fail to imatinib at the first year of treatment may be late responders. Not all patients should change therapy, if they have not reached MMR at 12 months. Molecular response at 3 at 6 months might guide the decision to switch TKI, but patient’s comorbidities, possibility of discontinuation and cost of therapy should also be considered.

PB1831
PREDICTIVE PARAMETERS FOR IMATINIB FAILURE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background: Development of tyrosine kinase inhibitors (TKIs) has significantly changed natural course of chronic myeloid leukemia (CML) and increased 10 year overall survival from 10-20% to 80-90%. Until recently, imatinib was the standard first-line treatment in CML. In 2013, nilotinib and dasatinib were approved as alternative front-line options. However, none of three TKI has been shown to have a clear survival advantage so this raised a debate on treatment selection. The early identification of patients expecting poor outcome is crucial for offering an alternative TKI regimen.

Aims: To analyze predictive parameters for Imatinib response as first-line treatment of CML patients.

Methods: The study was conducted on 168 consecutive patients with chronic phase of Ph+ CML who were diagnosed and treated at single university hospital from December 2000-January 2015. Following data were analyzed in terms of treatment response to Imatinib: demographic characteristics; currently used prognostic scores (Sokal, Hasford, EUTOS); liver and spleen size; laboratory parameters; incidence of comorbidities analyzed by three scores (ACE 27, HCl-CI, SCIRS); occurrence of second malignancies; conventional cytogenetic parameters; time to progression, duration of therapy, cytogenetic responses, overall survival (OS) and outcome.

Results: The mean age at diagnosis was 48±14.4 years (range: 18-74) with 87.5% of patients>65 years. The OS at 5 and 10 years was 97% and 91% respectively. Overall response to imatinib treatment was as the follows: 131 patients (78.8%) achieved CCyR (93.3%) and the remaining patients (6.7%) had minorCyR, 16 patients (9.5%) had no cytogenetic response, 2 patients (1.2%) had hepatic toxicity verified by liver biopsy in the first six months of Imatinib treatment and 1 patient (0.6%) was lost from follow-up. After achievement of CCyR, 25 patients (19%) had a progression of disease by losing CCyR or developing AP/BCR. Median time to progression was 24 months (range 12-102). After the median follow up of 87 months in 61 patients (36.3%), the Imatinib failure was verified. All three prognostic scores (Sokal, Hasford, EUTOS), age, gender, hemoglobin level, leukocyte and platelet count, splenomegaly, eosinophils and basophils in peripheral blood were not found to be statistically significant for the Imatinib failure. Cox regression analysis identified hepatomegaly (p=0.001), leukocyteosis100x109/l (p=0.001), blood blasts>1% (p=0.002) and presence of additional cytogenetic aberrations (ACAs) (p=0.002) as a predictors of Imatinib failure. Accordingly, we assigned risk scores based on hazard ratios (HR) to hepatomegaly (HR=4.089; 2 points), leukocytosis >10x10⁹/l (HR=3.158; 1 point), blasts in peripheral blood >1% (HR=2.912; 1 point), and presence of ACAs (HR=11.110; 2 points). A final 3-tiered prognostic model named IMA-FAIL was thus developed, as low (score 0), intermediate (score 1-3), and high risk (score ≥4), according to which imatinib failure had 17% (8/47) of patients in low, 34.9% (30/88) in intermediate and 76.7% (23/30) in high risk group (HR=3.973, 95% CI for HR 2.257-7.053, p<0.001). In addition, presence of comorbidities as well occurrence of second malignancy were not predictors for Imatinib failure.

Summary/Conclusions: Hematologists are facing with challenge of making decision which TKI to choose upfront with increasing a chance to achieve best possible response. The new score allows better selection of patients who are suitable for treatment with Imatinib and may guide the clinical decision for front-line treatment of CML.

PB1832
A MULTICENTRE AUDIT OF SYMPTOMS AND QUALITY OF LIFE IN IRISH CML PATIENTS ON TYROSINE KINASE INHIBITORS

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Background: The development of tyrosine kinase inhibitors (TKIs) over the last 20 years has dramatically improved the outcomes for patients with every stage of chronic myeloid leukaemia (CML). Since the approval of the first TKI, imatinib, in 2001, there are now currently 5 oral TKIs available. Three are approved for frontline use (imatinib, dasatinib and nilotinib) and 2 others (bosutinib and ponatinib) approved for intolerance or failure of prior TKI. Because CML patients may require multiple TKIs in their lifetime, it is necessary to consider not only differences in potency and progression-free survival, but also TKI induced toxicity and quality of life (QOL) when choosing a TKI.

Aims: The aim of this audit was to determine the impact of TKIs on symptom burden and QOL in patients currently on TKIs in centres in Ireland, using the MD Anderson Symptom Inventory (MDASI) tool.

Methods: Across 7 centres in Ireland, a total of 87 CML patients currently on TKIs were identified. The mean age was 60yrs with an equal sex distribution (44 male, 43 female). All of these patients were in chronic phase. 79% of patients were in MMR (major molecular remission) at the time of survey. 53 patients were on imatinib, 19 patients on nilotinib, 13 on dasatinib and 2 on bosutinib. Patients from the 7 centres were surveyed at varying time periods between July 2015 and Feb 2017. Patients were contacted by phone. Symptom burden and QOL were assessed using the MD Anderson Symptom Inventory, modified to include 13 symptom items, as well as 6 interference items. The questionnaire took on average 5mins to complete and asked patients to rate their symptoms on a scale of 1-10 as experienced over the preceding 24 hours.
Results: Of the 87 patients surveyed, the most commonly prevailing symptoms were fatigued (72.4%), peripheral oedema (48.3%), disturbed sleep (46%), myalgia (43.7%) and dry mouth (39.1%). The least common symptoms were nausea (20.7%) and vomiting (6.9%). Almost half (49.4%) of patients reported at least 1 severe side effect (a score of 7 or more). The most severe side effects were drowsiness (mean score 6.3), myalgia (mean score 6), fatigue, nausea and vomiting (mean score 5.7 each). There was no significant difference in frequency or severity of symptoms or in QOL compared to patients on imatinib. Despite excellent survival results obtained with TKIs since 2001, an emphasis needs to be placed on symptom burden and QOL. The potential tinib. Despite excellent survival results obtained with TKIs since 2001, an emphasis needs to be placed on symptom burden and QOL. The potential tinib. Despite excellent survival results obtained with TKIs since 2001, an emphasis needs to be placed on symptom burden and QOL. The potential tinib. Despite excellent survival results obtained with TKIs since 2001, an emphasis needs to be placed on symptom burden and QOL. The potential tinib. Despite excellent survival results obtained with TKIs since 2001, an emphasis needs to be placed on symptom burden and QOL. The potential...
Responses and Survival of Patients with Chronic Myeloid Leukemia Initially Treated with Imatinib: 11 Year Experience of a Teaching Hospital

Methods: This cross-sectional study comprised 85 patients with CML in chronic phase, treated with imatinib, at the Clinical Center of the University of Sarajevo, 2Faculty of Natural Sciences, University of Sarajevo, 3Hematology, Clinical Center Zenica, Sarajevo, 4Hematology, University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina.

Background: Tyrosine Kinase Inhibitors (TKIs) have the potential to cure CML. The aim of this study was to evaluate the efficacy and outcome of imatinib versus nilotinib in real life conditions.

Results: The median age at diagnosis of the patients was 49 years (range: 14-74). The median follow-up was 11 years (range: 2-18 years). The median duration of imatinib therapy is 66 months (range: 1 to 11 years). The proportion of CCyR and MMR at 24 months was higher in patients on first-line nilotinib compared to patients on first-line imatinib, respectively. Rate of death was similar in both studied groups (20/118 vs 22/118). When we analysed delayed treatment at 24 months, CCyR for patients who received imatinib immediately who waited 6-13 months and more than 13 months, was 74% vs 64% vs 40%, respectively. Regarding nilotinib treatment at 24 months, patients on 1st line immediate nilotinib vs 1st line delayed nilotinib achieved 83% vs 77% for CCyR and 78% vs 69% for MMR, respectively.

Summary/Conclusions: Our results after 11 years of follow up suggest that nilotinib demonstrated improved efficacy over imatinib. Achievement of CCyR and MMR at 24 months was higher in patients on front-line nilotinib. Patients who waited for therapy had optimal response regardless the wait period on nilotinib treatment.

The Influence of Age on Treatment Outcome of Patients with Chronic Myeloid Leukemia Receiving Frontline Imatinib

Methods: This study included 85 patients with CML treated with imatinib, at the Clinical Center of the University of Sarajevo, 2Faculty of Natural Sciences, University of Sarajevo, 3Hematology, Clinical Center Zenica, Sarajevo, 4Hematology, University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina.

Background: The tyrosine kinase inhibitor (TKI) imatinib was the first targeted therapy for patients with chronic-phase chronic myeloid leukemia (CP-CML), and it significantly improved survival, CCyR and MMR. Nilotinib was introduced in 2011 as front- and second-line therapy for newly diagnosed as well as patients who waited for TKI treatment for a long time.

Aims: In this study we compared the long-term real life clinical outcomes (OS, CCyR and MMR) of patients receiving front-line imatinib and front-line nilotinib in Bosnia and Herzegovina in the period from 08/2005 to 08/2016, categorized based on delayed start of therapy.

Summary/Conclusions: Our results after 11 years of follow up suggest that nilotinib demonstrated improved efficacy over imatinib. Achievement of CCyR and MMR at 24 months was higher in patients on front-line nilotinib. Patients who waited for therapy had optimal response regardless the wait period on nilotinib treatment.
adverse event (AEs), the 5-year event-free survival (EFS) and 5-year overall survival (OS) were all evaluated. Clinical features of the patients in different age groups are summarized in Table 1.

**Results:** The patient cohort consisted of 94 patients with median age of 53.4 years (range 18–78), with a slight predominance of females of 53.2%. There were more patients with intermediate and high Sokal scores in the EP group than in the groups MA and YA (p<0.001). To the contrary of that, most patients with high EUTOS score were observed in the group YA compared to MA and EP groups (p<0.001). The three groups were balanced regarding Euro score. The median duration of imatinib therapy was the longest in MA group (61.4 months vs 40.6 months in YA and 38.2 months in EP patients p<0.001). Furthermore, median follow-up duration was also the longest in MA group (64.3 months vs 48.5 months in YA and 44.7 months in EP patients p<0.001).

The rates of complete cytogenetic response (CCyR) were similar in all three analysed groups (80.6% in YA, 86.5% in MA and 75.9% in EP, p=0.328) while rate of major molecular response was the highest in the MA group (83.3% vs 63.3% in YA and 57.1% in EL, p=0.001). The percentages of patients who switched to second-generation TKIs were similar in all three groups (36.7% in YA vs 30% in MA vs 32.1% in EP, p=0.559). There were the most of non-hematological AEs all grades in EP group (25% vs 13.3% in YA and 13.8% in MA, p=0.005). Hematological AEs also were common in EP group but not statistically significant (17.8% vs 10% in YA and in 12.1% in MA, p=0.156). The 5-years EFS in the MA group (88% (95%CI 82.1-96.9)) was significantly higher than in YA group (65.3% (95%CI 59.1-71.8)) and in EP group (60.2% (95% CI 49.5-73.7)). The 5-years OS in the EP group (74.7% (95%CI 65.9-83.9)) was significantly lower than in YA group (93.1% (95%CI 87.2-99.5)) and in MA group (90.8% (95% CI 85.8-97.8)). The number of deaths, both CML related or not, was the largest in the EP group (25% vs 13.3% in YA and 13.8% in MA, p<0.001).

Table 1. Clinical features of the patients in different age groups.

**Summary/Conclusions:** Results of this study indicate that age at diagnosis impacts the course of chronic myeloid leukemia treated with imatinib. The best clinical outcomes have middle age patients in terms of the highest rates achieved optimal therapeutic response and longer survival without events and overall survival. The degree of therapeutic responds in the elderly is comparable with that observed in younger patients, but the presence of comorbidity and more frequent occurrence of adverse events were affecting relatively lower overall survival.

Although it might be expected that younger patient population has a better clinical outcome than patients middle age, a possible cause of poor outcomes is probably a late diagnosis at an advanced stage of the disease.

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**Enzymopathies, membranopathies and other anemias**

**PB1839**

**CHARACTERIZATION OF HEMATOPOIETIC SAMPLES FROM PYRUVATE KINASE DEFICIENCY PATIENTS**


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**Background:** Pyruvate Kinase Deficiency (PKD) is an autosomal recessive disease caused by mutations in the PKLR gene. PKD produces chronic non-sphero- chytic hemolytic anemia, which can be fatal during early childhood and may result in lifelong transfusion dependence that in some instances persists despite therapeutic splenectomy. Although not considered a standard-of-care, allogenetic bone marrow transplantation has been curative in a limited number of cases. These findings, and the evidence that PKLR gene mutations are likely the sole etiology of the disorder, make PKD a candidate for gene therapy. Our lab has developed a therapeutic Orphan Drug lentiviral product (EMA: EU/3/14/1330; FDA: DRU-2016-5168) for the treatment of PKD and is working to develop an efficient and safe gene therapy clinical trial for the treatment of PKD.

**Aims:** In order to improve this new treatment, a more deep knowledge of the disease and its associated pathophysiology is necessary.

**Methods:** To characterize the hematopoietic profile of this disease, we have standardized flow cytometry protocols to perform both a qualitative and quantitative study of different population subsets. These included subsets of the hematopoietic stem cell compartment, erythroid progenitors, reticulocytes, mature erythrocytes and other mature lineages. Human routine samples consisted of peripheral blood, bone marrow and cord blood from PKD patients. In addition, xenogenic engraftment studies in immunodeficient (NSG) mice were also performed.

**Results:** Flow cytometry studies showed a clear imbalance in the erythroid populations. On the other hand, human PKD progenitors were able to engraft into NSG mice demonstrating that the disease does not likely impair hematopoietic stem cell capabilities.

**Summary/Conclusions:** Pyruvate Kinase Deficiency (PKD) is an autosomal recessive disease caused by mutations in the PKLR gene. Our lab has recently developed a therapeutic Orphan Drug lentiviral product for the treatment of PKD. In order to improve this new treatment, we are also working to deep into the knowledge of the disease and its associated pathophysiology. Flow cytom- etry studies have shown a clear imbalance in the erythroid populations. Functionally, results in NSG mice we have demonstrated that the disease does not likely impair hematopoietic stem cell capabilities.

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**PB1840**

**OSMOTIC GRADIENT EKTACYTOMETRY: A VALUABLE SCREENING TEST FOR HEREDITARY SPHEROCYTOSIS AND OTHER RED BLOOD CELL MEMBRANE DISORDERS**

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**Background:** Red blood cell (RBC) membrane disorders constitute one of the major causes of chronic hereditary hemolytic anemia. Main RBC membrane disorders, namely hereditary spherocytosis (HS), hereditary elliptocytosis (HE) and hereditary stomatocytosis (HST), alter membrane cohesion, membrane mechanical stability, and RBC volume, respectively. As a consequence, RBC deformability is compromised leading to their premature removal from circulation, manifested as hemolytic anemia. New generation osmotic gradient ektacytometry has become a powerful procedure to measure red cell membrane deformability and therefore for the diagnosis of red blood cell membrane disorders.

**Aims:** The aim of this study is to evaluate osmotic gradient ektacytometry as an adequate assay to perform screening of membranopathies, focusing on the differential diagnosis between HS and non-spherocytic membrane defects such as HE and dHST.

**Methods:** A total of 75 patients with chronic hemolytic anemia oriented as hereditary RBC membrane disorders (hemoglobin disorders discarded and negative Coombs test) were included during a period comprised between January 2015 and August 2016. Normal controls were obtained from blood donors. Osmotic gradient ektacytometry was performed using the osmoscan module of the Laser-assisted Optical Rotational Deformability Cell Analyzer: LoRRaC MaxSis (RR Mechatronics). Evaluation of osmocan parameters...
robustness for HS diagnosis was performed using the receiver operating characteristic (ROC) curve analysis. The optimal cut-off was determined as the one with the highest likelihood ratio. Statistic analysis was operated with GraphPad Prism.

Results: Specific patterns of osmotic LoRRCa MaxSis were observed for each individual membranopathy. All HS curves were bell shaped but two different profiles were identified both presenting increased Omin, and decreased Emax and AUC. HE curves showed a characteristic trapezoidal shape with a decreased Emax, Omax and AUC. dHSt curve was bell shaped with a specific decrease in Othyper and a slight increase in Elmin. Reference ranges for each osmotic parameter were established with 171 healthy subjects and compared with the values of the parameters obtained from the different RBC membrane disorders. ROC curve analysis was performed for HS and each one of the non-HS groups separately. The results determined that Elmax was the parameter that better separated HS from normal controls and dHSt, while the Omin was the best to separate HS from HE. The optimal Elmax cut-off to differentiate HS from normal controls was Emax (sensitivity 98.40%, specificity 99.42%), while the optimal Omin cut-off to differentiate HS from HE was >159.0 (sensitivity 95.38%, specificity 85.71%). Expressing the results as% of variation in relation to the mean of our normal controls, the best combination of parameters for HS diagnosis would be Elmax <3% and Omin >5.2%. This combination of parameters reveals Emax and Omin (5.2%) was used as criteria to classify all the 246 samples included in the present study, and the result showed 62 samples detected as HS and 184 as no-HS. Of the 62 patients identified as HS, 61 were real HS (specificity 98.38%) and 1 was an HE. On the other hand, 4 HS patients were identified as non-HS (sensitivity 93.85%).

Summary/Conclusions: We can conclude that, the inclusion of LoRRCa osmotic as a screening test in RBC membrane diagnostic workflow will signify an important advance for the accurate diagnosis of HS patients, as well as for the identification of HE and specially dHSi patients.

PB1841
RARE RED BLOOD CELL ENZYMOPATHIES INDUCED CHRONIC NONSpherocytic HEMOLYTIC ANEMIA: NEXT GENERATION SEQUENCING BASED MOLECULAR DIAGNOSIS
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Background: Red blood cell enzymopathies are mostly inherited autosomal recessive monogenic disorders. Mutations in the genes encoding red blood cell enzymes could lead to chronic nonspherocytic hemolytic anemia (CNSHA). The clinical manifestations are jaundice, cholelithiasis, splenomegaly, with usually normocytic normochromic hemolytic anemia. Phenotypes vary from having fully compensated hemolysis (without anemia) to severe hemolytic anemia requiring regular transfusions. Definitive diagnosis is difficult when biochemical test results are not consistent/fail to identify defects. Molecular diagnosis by gene-by-gene approach is expensive, time consuming and cumbersome as testing for multiple genes is required. Aims: Use of targeted resequencing can expedite the molecular diagnosis where the cause for hemolysis remains unexplained after routine laboratory tests. Methods: Ten patients with clinical and laboratory evidence suggestive of hemolytic anemia were enrolled. Various biochemical and molecular tests were used to exclude Glucose-6-phosphate dehydrogenase (G6PD) deficiency, thalassemias, hemoglobinopathies, autoimmune hemolytic anemia, hereditary spherocytosis and pyruvate kinase deficiency. Common G6PD and PKLR variants were excluded by molecular tests. Family history was negative in all the cases. Libraries were prepared using TruSight One sequencing panel and sequenced on MiSeq™ Sequencing System. MiSeq Reporter™ and Variation Studio™ v2.1 were used for analysis, classification, and reporting of germline variants.

Results: Two patients with G6PD deficiency, six patients with pyruvate kinase (PKLR) deficiency and two patients with Glucose-6-phosphate isomerase (GPI) deficiency were found. Unexpected pyruvate kinase defects were found on target resequencing for six patients. Pyruvate Kinase (PK) enzyme activity assay were within normal limits in all these cases. All the mutations were predicted deleterious by PolyPhen/ SIFT/ Provean/ mutpred and Mutationtaster. Mutations were validated in the parents/siblings (where available) to prove the molecular inheritance.

Summary/Conclusions: Unexpected PK deficiency were found after next generation sequencing analysis in the patients where PK enzyme levels were within normal limits. PK deficiency may be missed by conventional testing approaches. Our data demonstrates the clinical utility of next generation sequencing for molecular diagnosis. Timely detection of the cause in our patient is likely to prevent unnecessary testing, diagnose and treat patients with PK deficiency, if required, but therapeutically as well. A splenectomy (performed at appropriate age) can ameliorate the anemia in such patients and can eliminate need for transfusions in those that need them.

PB1842
COMPARISON STUDY OF THE EOSIN-5'-MALEIMIDE BINDING TEST, OSMOTIC FRAGILITY TEST AND CRYOHEMOLYSIS TEST IN THE DIAGNOSIS OF HEREDITARY SPHEROCYTOSIS
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Background: The primary lesion in HS is loss of membran surface area due to defects of the membran protein. Cryohemolysis test and osmotic fragility (OF) test are used for screening. However no test for HS is 100% reliable. The eosin-5'-maleimide (EMA) binding test based on flow cytometry. Eighty percent of the fluorescent-labelled EMA binds to band 3 protein which is lost in HS due to protein 4.1, spectrin and ankyrin deficiency. Thus these measurement of the fluorescent EMA tests detects all the different forms of HS.

Aims: In this study we aimed to evaluate the concordance of EMA binding test with other diagnostic parameters for HS.

Methods: The patients with HS were diagnosed according to clinical findings for hemolytic anemia, splenomegaly and spherocytes in peripheral blood. Hemogram, reticulocyte count, total/direct bilirubin, spherocytes in blood smear (BS), EMA binding test, OF test, and cryohemolysis test were obtained from patients and control groups. Correlation between EMA, OF and cryohemolysis tests were evaluated.

Results: Twenty-five male, 17 female HS patients aged between 1.0-19.0 years and 38 male, 47 female healthy controls were evaluated. There were no differences between both groups in terms of age and sex (Table 1). The median (range) values of hemoglobin (%), reticulocyte count (%), mean corpuscular volume (fL), MCHC (%), and total bilirubin level were seen in Table 1. Besides MCV values there were general differences between both groups (Table 1). The median MCF of HS patients was significantly lower than that of healthy controls while cryohemolysis and osmotic fragility were higher in HS patients than healthy controls (Table1). There were moderate concordence between cryohemolysis and EMA test (r=0.355, p<0.001). The sensitivity of EMA was 92.86%, specificity was 82.25%, PPV was%72.22, NPV was%95.89. EAMA was superior diagnostic test to osmotic fragility. (sensitivity: 83.33, specificity, 76.47, PPV:63.84 and NPV:90.28). The sensitivity of cryohemolysis test was 90.48%, specificity was 94.12%, PPV was88.37, NPV was%95.24.

Table 1. Comparison of Clinical and Laboratory Findings in Hereditary Spherocytosis groups and Healthy Controls

<table>
<thead>
<tr>
<th></th>
<th>Healthy Controls</th>
<th>Healthy Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, M/F</td>
<td>22</td>
<td>36</td>
</tr>
<tr>
<td>Age, years</td>
<td>1.0-19.0</td>
<td>1.0-38.0</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>78.7</td>
<td>74.1</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>31.0-37.0</td>
<td>32.0-37.0</td>
</tr>
<tr>
<td>MCH (%)</td>
<td>31.0-37.0</td>
<td>32.0-37.0</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Direct bilirubin</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Hemoglobin (%)</td>
<td>12.0-20.0</td>
<td>12.0-20.0</td>
</tr>
<tr>
<td>Reticulocyte</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>EMA (fL)</td>
<td>61.6 (3.8-34.1)</td>
<td>61.6 (3.8-34.1)</td>
</tr>
<tr>
<td>OF (L)</td>
<td>32.0 (27-37.0)</td>
<td>32.0 (27-37.0)</td>
</tr>
<tr>
<td>EMA (fL)</td>
<td>10.1 (6.0-14.0)</td>
<td>10.1 (6.0-14.0)</td>
</tr>
<tr>
<td>OSM (fL)</td>
<td>37.8 (10.0-48.0)</td>
<td>37.8 (10.0-48.0)</td>
</tr>
<tr>
<td>Cryo (fL)</td>
<td>10.1 (6.0-14.0)</td>
<td>10.1 (6.0-14.0)</td>
</tr>
</tbody>
</table>

Summary/Conclusions: In this study EMA-FC was more sensitive and specificity than osmotic fragility. However specificity and PPV of cryohemolysis was higher than other test. Also we showed moderate concordance cryohemolysis and EMA test. Although high sensitivity and specificity of EMA test there were need to use other tests together with family history of patient, physical examination, evaluation of blood smear and several tests for HS diagnosis.

PB1843
ADVANCES IN DIAGNOSIS OF HEREDITARY HEMOLYTIC ANEMIAS: THERMOGRAVIMETRY COUPLED WITH CHEMOMETRICS
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Background: The differential diagnosis of hereditary hemolytic anemia is generally carried out by applying different diagnostic protocols depending on the specific congenital erythrocyte defects. Thermogravimetric analysis (TGA) coupled with chemometrics has recently been proposed as a rapid and cost effective diagnostic tool for β-thalassemia screening. This model, consisting of Partial Least Square-Discriminant Analysis (PLS-DA), permitted the discrimination of thalassemic patients and healthy individuals, using thermogravimetric curves of blood samples [1].

Aims: In this study, the capability of thermogravimetric in correlation with a mut-
tivariate statistical analysis was investigated for the screening of hereditary hemolytic anemias due to different erythrocyte defects.

**Methods:** Whole blood samples collected in K2EDTA were obtained, after informed consent, from patients suffering from congenital hemolytic anemias and were analyzed using the thermobalance TGT (Perkin Elmer) without any pretreatment and the resulting curves were compared with those of healthy individuals. Two groups of hereditary hemolytic anemias were considered: the hemoglobinopathies (sickle cell anemia and thalassemia) and the erythrocyte membrane defects (hereditary elliptocytosis and hereditary spherocytosis).

**Results:** The characteristic profile of the blood sample thermal decomposition and the first derivative (DTG) of the TG curve showed that blood 2 samples from anemic patients were clearly distinguished from those of healthy individuals as a result of different amounts of water and corpuscular fraction. The chemometric approach based on Principal Components Analysis (PCA) allowed a quick identification of differences between healthy and anemic patients in order to point out a model of prediction in patients with heterogeneous congenital hemolytic disorders.

**Summary/Conclusions:** The achieved results allow to consider the coupling TGA/Chemometrics as a promising diagnostic approach to provide a high-throughput and sensitive tool to obtain an early detection of hereditary hemolytic anemias using only a few microliters of blood without any pretreatment and with an hour of analysis time.

PB1844

**DEVELOPMENT OF A POINT-SCORING SYSTEM FOR EARLY DIAGNOSTIC TESTING IN GAUCHER DISEASE: APPLICATION OF FINDINGS FROM THE GAUCHER EARLIER DIAGNOSIS CONSENSUS DELPHI INITIATIVE**

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**Background:** In the Western hemisphere, Gaucher disease (GD) type 1 is the most common GD phenotype, but the prevalence of GD type 3 is increasing. Early diagnosis of GD is of utmost importance, as symptoms are highly variable in different GD phenotypes. The aim of this study was to develop a point-scoring system (PSS) suitable for use across clinical specialties, that provides guidance based on patients’ presenting signs as to whether GD diagnostic testing is appropriate.

**Methods:** An anonymous three-round Delphi process, conducted among a global panel of 22 expert physicians, established consensus on which signs and co-variables may be important in early GD type 1 and, separately, in early GD type 3. In round 1, free-text responses provided by the panel were categorized by the independent administrator. This categorization was checked and consolidated into summary factors by the non-voting co-chairs. In round 2, the factors were rated for importance by the panel using a 5-point Likert scale (1 = not important, 3 = important, 5 = extremely important). Any factors assigned an importance score of ≥3 by >75% of respondents were then rated for agreement in round 3, using a 5-point Likert scale (1 = strongly disagree, 3 = neither agree nor disagree, 5 = strongly agree). Consensus was defined as a score of ≥4 by >67% of respondents. Factors meeting this threshold were classified as major; all other factors were classified as minor. The co-chairs defined value ranges corresponding to mild, moderate or severe forms of five of the major signs of GD (anaemia, hepatomegaly, hyperferritinaemia, splenomegaly and thrombocytopenia). Panel members indicated whether they regarded each range as consistent with a GD diagnosis. This information was used in combination with the classifications of signs and co-variables as major or minor to create a prototype PSS.

**Results:** The final prototype PSS incorporated 35 factors, 21 classified as major, and 14 classified as minor. The panel was divided into whether severe anaemia, hepatomegaly, hyperferritinaemia and severe thrombocytopenia were consistent with a GD diagnosis, so these were assigned a score of 1. All minor signs and co-variables were assigned a score of 0.5.

**Summary/Conclusions:** The developed PSS will be validated with retrospective patient data. Total patient scores based on presenting signs and co-variables will be used to determine empirically a minimum threshold score that captures positive tests for GD. Abstract submitted on behalf of the GED-C panel and the EHA Scientific Working Group 'Quality of Life and Symptoms'. Administration of the GED-C initiative was funded by unrestricted educational grants from Shire International GmbH.

PB1845

**REGIONAL DISTRIBUTION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN TURKEY AND EVALUATION OF CLINICAL FINDINGS**

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**Background:** Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common inherited enzyme deficiency, that affects more than 400 million people around the world with more than 300 variants. According to data by the World Health Organization which was published in 1989, 7.5% of people in the world have at least one gene G6PD deficiency and this ratio is the highest in sub-Saharan Africa and Southeast Asia (15-26%). This ratio is in the range of 0.5-2.9% in Turkey, as United States and the neighboring countries to Mediterranean Sea. The epidemiological studies about G6PD deficiency in Turkey were mostly regional or limited to a city.

**Aims:** We aimed to evaluate in terms of regional distribution and clinical features of G6PD deficiency by screening the patients who applied for soldier recruitment.

**Methods:** The patients who applied for soldier recruitment between January and March 2016, were analyzed retrospectively. Patients, who were diagnosed G6PD deficiency were scanned by using hospital patient information system. The patients’ ages, the cities they lived, complaints and the stories of them were questioned. Complete blood count, serum AST, LDH, total and direct bilirubin levels of all the cases in the study were recorded. G6PD levels were measured by quantitative spectrophotometric methods in biochemistry laboratory. The World Health Organization (WHO) is divided G6PD enzyme deficiency into five classes based on enzyme activity levels and clinical findings.

**Results:** The distribution of the cases where the cases were living, was given on the map in Figure 1. Patients’ average age, hemoglobin, and G6PD levels were 26.42±4.62, 14.68±1.51, and 0.86±0.391 respectively. According to clinical history of patients prior to diagnosis, 29 patients (20.7%) were diagnosed after acute hemolytic episodes. Of these patients 23, 4, 2, had hemolytic episodes due to drug, infection, chemical respectively. Subsequently, 78 (54.5%) and 27 (18.9%) of the remaining patients were diagnosed G6PD deficiency by excluding examinations due to hemolysis after favism and prolonged neonatal jaundice respectively. 6 patients (4.3%) were diagnosed of G6PD deficiency by screening because of family history, but they didn’t have any hemolytic episodes before. After the patients evaluated with their clinical history and hemolysis findings; 6 patients (4.3%), who had chronic hemolysis, was considered compatible with Class I variant. 128 cases were considered as Class II variants.

**Summary/Conclusions:** G6PD enzyme deficiency in Turkey is seen most frequently in the Mediterranean region and the prevalence of G6PD deficiency in Central Anatolia and Aegean regions was found to be over the Turkey average (2%). Nearly half of the patients had hemolytic episodes due to favism. It is followed by hemolysis due to neonatal hyperbilirubinemia and drugs. 128 (91.4%) patients who had severe G6PD deficiency with intermittent hemolysis, were considered as Class II variants.
CHARACTERISTICS AND MANAGEMENT OF AUTOIMMUNE HEMOLYTIC ANEMIA: A SINGLE CENTER STUDY WITH 32 CASES
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Background: Autoimmune hemolytic anemia (AIHA) is characterized by red blood cell destruction mediated with autoantibodies against to RBC antigens. Most common type is warm AIHA which can be either idiopathic or secondary to underlying disorders with immune disturbance. Determining the optimal therapy is a challenge because of insufficient data from prospective controlled trials.

Aims: To evaluate the clinical characteristics, treatment responses and outcomes of our AIHA patients.

Methods: The clinical data of 32 patients with AIHA diagnosed and treated in our center between 2008 and 2016 were retrospectively analyzed.

Results: Median age on diagnosis of AIHA was 45 years (range:20-74). Male/female ratio was 1/1.3. 24 of 32 patients (75%) had primary AIHA and 8 (25%) had secondary AIHA with underlying disorders as SLE in 2 patients, mixed connection tissue disease (MCTD) in 2, psoriatic arthritis in 1, chronic lymphocytic leukemia (CLL) in 1, marginal zone lymphoma in 1 and, chronic HCV infection in 1. Median Hemoglobin (Hb) level was 7.4 g/dl and 5 patients also had thrombocytopenia (<150000) beside hemolytic anemia. Mean LDH level was 544, indirect bilirubin was 2.7, reticulocyte was 11.3%. 18/32 patients (56%) required transfusion. In all patients who required treatment (94%) corticosteroids were the first-line therapy with an initial response rate of 93%. Median steroid duration was 3 months range between 1.5 to 96 months. Relapse was occurred in 15 of 30 patients who received steroid (50%) with the median time to relapse (TTR) of 12 months (range:5-72 months). 11/30 patients (37%) required second-line therapy; seven had undergone splenectomy, three received rituximab, and one received danasum. All of the patients who undergo splenectomy had CR in first month and relapse after splenectomy was seen in 5/7 patients (71%) with a median duration of 60 months. Of 3 patients who were treated with standard dose of Rituximab; two achieved CR and one did not achieve any response. One of two rituximab-treated patients relapsed at 26. and 60. months and re-treated by rituximab; still following with CR for 16 months.

Summary/Conclusions: Although corticosteroids are the first choice of initial treatment of AIHA; most of the patients relapse at follow up. Steroid dependency and intolerance are also challenging. Splenectomy is still a considerable option for second-line therapy because of its high response rates and long remission durations. Rituximab is the other effective second-line therapy option with similar response rates to splenectomy. Until prospective studies will be performed, retrospective data would help the clinicians to choose best treatment algorithm for AIHA.

THE IMPACT OF THE REORGANIZATION OF THE PATIENT CARE PROCESS FOR GAUCHER DISEASE IN HEALTH SYSTEM
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Background: Gaucher disease (GD) is a multisystemic disease of lysosomal storage that is caused by deficient activity of the glucocerebrosidase enzyme resulting from a recessive autosomal hereditary mutation in the β-glucocerebrosidase gene. The accumulation of glucocerebrosidase in the lysomes damages the hematological, skeletal, and nervous systems and leads to three varieties of the disease: type 1, which is non-neuropathic, and types 2 and 3, which are neuropathic. In Mexico, the process by which patients with lysosomal disease are cared for was recently formalized by the Clínicas de Referencia Nacional y Grupos de Expertos en Enfermedades Líbosomales (National Reference Clinics and Expert Groups on Lysosomal Diseases [EGLDs]), who created the Guías de Práctica Clínica (Clinical Practice Guidelines) for GD.

Aims: To evaluate the results obtained for 39 patients diagnosed with type 1GD (25 women and 14 men) through the National Reference Clinics and EGLDs.

Methods: The clinical case of 39 patients was analyzed and punctual mutation of the β-glucocerebrosidase gene was determined. The patients were treated with imiglucerase enzyme at 60 U/Ikg every 14 days. The enzymatic activity of the β-glucocerebrosidase and the chitotriosidase was determined. We determined concentration of hemoglobin and platelets. The degree of hepatosplenomegaly, bone density and skeletal pain was evaluated.

Results: Four of the 39 patients were found to have been incorrectly diagnosed with GD, the remaining 35 patients completed the treatment goals, which included remission from hepatosplenomegaly, splenomegaly, and skeletal pain. Additionally, increases in the hemoglobin and platelent concentration and bone mineralization were achieved, thereby attaining the patients’ therapeutic goals, reducing the therapeutic dose required, and achieving the expected impacts on their health.

Summary/Conclusions: This reorganization of patient care successfully reduced complications, improved care, and optimized the use of resources and costs of GD treatment.
**Gene therapy, cellular immunotherapy and vaccination**

**PB1849**

**DEMONSTRATION OF FUNCTIONAL SIMILARITY OF PROPOSED BIOSIMILAR ABP 798 TO RITUXIMAB**

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**Background:** Proposed biosimilars undergo comprehensive structural and functional characterization before they can be studied in confirmatory clinical trials. ABP 798 is being developed as a biosimilar to rituximab. The originator is approved for treatment of non-Hodgkin's lymphoma, chronic lymphocytic leukemia, severe rheumatoid arthritis, granulomatosis with polyangiitis, and microscopic polyangiitis.

**Aims:** ABP 798 was compared with rituximab sourced from the European Union (EU). Quality attributes assessed included binding properties (CD20, C1q, FcRn, and Fc receptors), antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and induction of apoptosis.

**Methods:** Binding of ABP 798 and rituximab to the CD20 antigen was characterized using a cell-based CD20 binding assay utilizing the human B-lymphoblastoid, WIL2-S, cell line. A direct binding ELISA was used to assess the binding of the Fc domain of ABP 798 to C1q. Binding of the Fc moiety of ABP 798 and rituximab to FcγRia, FcγRil, FcγRlb, and FcγRilla (185V) were evaluated in AlphaLISA® competitive binding assays. Binding to FcRn was evaluated by an AlphaScreen® competitive binding assay. ADCC activity was evaluated in a functional cell-based assay, with CD20-expressing WIL2-S cells used as target cells and NK92-M1 cells, stably transfected with human CD16 (FcyRilla [185V]), as effecter cells. CDC activity was evaluated in a functional cell-based assay using a CD20 expressing human B-lymphoblastoid cell line and baby rabbit complement. Induction of apoptosis was assessed by measuring activation of caspase 3/7 in SU-DHL-4 cells, a CD20-expressing human B cell lymphoma cell line.

**Results:** Relative binding (%) was comparable between ABP 798 and rituximab (Table 1).

<table>
<thead>
<tr>
<th>Assay</th>
<th>ABP 798</th>
<th>Rituximab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotin/CD20</td>
<td>97</td>
<td>98</td>
</tr>
<tr>
<td>C1q</td>
<td>85-100</td>
<td>85-100</td>
</tr>
<tr>
<td>FcγRia</td>
<td>89-104</td>
<td>92-115</td>
</tr>
<tr>
<td>FcγRil</td>
<td>94-98</td>
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</tr>
<tr>
<td>FcγRlb</td>
<td>96-102</td>
<td>96-105</td>
</tr>
<tr>
<td>FcγRilla (185V)</td>
<td>80-96</td>
<td>87-97</td>
</tr>
</tbody>
</table>

The dose response profiles and relative activity for ADCC and CDC were similar (mean ADCC relative activity: ABP 798, 88%; rituximab, 86%; mean CDC relative potency: ABP 798, 103%; rituximab, 104%). The dose response profile for induction of caspase 3/7 was comparable between ABP 798 and rituximab.

**Summary/Conclusions:** The results presented here suggest that ABP 798 is similar to rituximab sourced in the EU in terms of biological activity across the range of tested functions. These results provide a firm foundation for further clinical development of ABP 798.

**PB1851**

**MYD88 IN PRAME GENE ACTIVATION**

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**Background:** PRAME is the most frequently expressed non-X-chromosomal cancer-testis gene in solid and hematological cancer. It is important, because PRAME often has a bad prognostic significance. In early studies was found that PRAME frequently coexpressed in translocation-harboring (like t(8;21), t(15;17) and t(9;22)) haematological diseases. Authors supposed that chimeric genes are activators of PRAME expression. But in large cases with normal karyotype PRAME is also expressed. Another reason for PRAME expression is promoter demethylisation. But demethylating agents cannot activate PRAME expression in hematological cells taken from healthy donor. So presence of chimeric genes and methylation status only are not enough to explain why PRAME can be expressed in high level. Wadelin et al. found that PRAME expression level was increased in cell during lipopolysaccharide-treatment conditions. Role of MYD88 in this process still be unknown.

**Aims:** To check if MYD88 participates in activating PRAME expression in leukemia cell lines.

**Methods:** Three cell lines were used for incubation with anti-PRAME antibody: chronic myeloid leukemia cell line K562 with high PRAME expression level (645%), acute monocytic leukemia cell line THP-1 with intermediate PRAME expression level (2.92% relative to ABL) and acute myeloid leukemia cell line NOMO-1 with low PRAME expression level (0.46%). All cell lines were incubated in RPMI 1640 with addition of LPS in final concentration 10 ng/ml. After 1 and 4 hours of incubation total RNA was extracted and PRAME and MYD88 expression levels were measured.

**Results:** After 1 and 4 hours of experiment in K562 cell line PRAME expression level was increased in 2.7 and 7 fold under control, respectively, and MYD88 expression level increased in 1,1 and 2.5 fold under control. In THP-1 line PRAME expression level was increased in 20 and 25 fold, respectively, and MYD88 expression level was increased in 5,5 and 6,5 fold. In cell line NOMO-1 PRAME expression level was increased in 10 fold after 1 hour and in 14 fold after 4 hours, and MYD88 expression level was increased in 2,4 and 3,2 fold after 1 and 4 hours of experiment, respectively. Strong correlation between MYD88 and PRAME expression levels was observed (Pearson correlation coefficient 0.98).

**Summary/Conclusions:** We conclude that LPS after binding with TLRs initiates activating signal to PRAME gene via MYD88.
Hematopoiesis, stem cells and microenvironment

PB1852

PD-1 IS HIGHLY EXPRESSED ON MEMORY T-CELL SUBSETS RESIDING IN BONE MARROW BUT NOT IN PE-RIPHERAL BLOOD IN HEALTHY INDIVIDUALS

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Background: Recently memory T lymphocytes were shown to be a highly heterogeneous cell compartment comprising different phenotypes, functional activities, gene expression profiles and survival capacities. Phenotypically due to the differentiation stage and functional activities memory CD8+ T cells can be divided into four different functional (Tem) subsets: central memory (Tcm), effector memory (Tem) and terminal effector (Tte) and reside in bone marrow (BM) as long-lived persistent T cells [Mahnke YD et al., 2013]. Programmed cell death protein 1 (PD-1) is well known as a negative immune regulator of T cells that has detrimental effects on anti-viral, anti-tumor immunity, mediates tissue tolerance to protect against immune-mediated tissue damage. Currently anti-PD1 immunotherapies are among the most effective anti-cancer immunotherapies available. PD1 pathway blockade is a key pathogenetic mechanism [Bousis MZ et al., 2014]. Understanding the influence of PD-1 pathway on memory T cells homeostasis in BM might be critical for improving treatment of patients with cancers and hematological malignancies, but is still not well understood.

Aims: To evaluate PD-1 expression on distinct memory T cell subsets in BM and PB of healthy donors.

Methods: The first portion of BM and a sample of PB were obtained from healthy donors (n=10, m=6, f=4) with age 37.5 (22-53) years old. Numbers of white blood cells (WBC) in BM and PB samples were evaluated by Sysmex XE-2100 hematology analyzer. 1*10^6 of WBC (excluded nucleated red blood cell) from BM and PB were stained using “lyse-wash-stain” standard protocol. The CD8-APC-Cy7, CCR7-PE-Cy7, CD28-PE, CD45R0-FITS, PD1-APC antibodies on cell membranes and 7-AAD were used for to discriminate living cells from BM and PB respectively.

Table 1.

<table>
<thead>
<tr>
<th>PD1 expression on cell subsets</th>
<th>7-AAD</th>
<th>CD8-APC-Cy7</th>
<th>CCR7-PE-Cy7</th>
<th>CD28-PE</th>
<th>CD45R0-FITS</th>
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</thead>
<tbody>
<tr>
<td>BM</td>
<td>p&lt;0.01</td>
<td>0.64±0.11</td>
<td>0.42±0.06</td>
<td>0.23±0.09</td>
<td>0.26±0.06</td>
</tr>
<tr>
<td>PB</td>
<td>p&lt;0.01</td>
<td>0.64±0.11</td>
<td>0.42±0.06</td>
<td>0.23±0.09</td>
<td>0.26±0.06</td>
</tr>
</tbody>
</table>

Summary/Conclusions: We found higher frequencies of PD-1 expressing memory BM T cells comparing to PB. This might point to the important roles of PD-1 in regulation of memory T cells homeostasis in BM. In physiological conditions PD-1 is thought to neutralize self-reactive naïve T cells that in its turn leads to restrain T cell activation and blockade the development of autoimmune in BM. On the other hand low expression of PD1 on T cells in PB can be explained by needs the opportunity for prompt reactivity with pathogens that also provide normal “robust control” and prevent developing of a disease.

PB1853

BONE MARROW STROMAL CELLS MAY HAVE GENETIC ABBERRATIONS AND ARE CAPABLE TO GAIN THEM IN A CULTURE

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Background: Stromal microenvironment plays a key role in the regulation of both normal hematopoiesis and its reconstitution after hematopoietic stem cell transplantation (HSCT). Recent data supports the idea that bone marrow stromal cells (BMSC) also have genetic aberrations and may tightly involved in the pathogenesis of HSCT complications. These findings justify the need for more detailed study of genetic aberrations in BMSC.

Aims: The aim of this study was to evaluate genetic aberrations in BMSC and check the ability to gain them in coculture system.

Methods: The interaction of BMSC with hematopoietic tumor cell lines bearing specific genetic aberrations (BCR-ABL fusion transcript for K-562 and JAK2 V617F mutation for Uke-1 cell line) was investigated in stromal cells harvested from 17 patients and 8 healthy donors. We performed cultivation of BMSC in coculture with tumor cells using semipermeable membrane plates with different pore size (0.4 μm and 3.0 μm) in order to exclude direct cell-to-cell contact. We looked also for existing specific genetic aberrations (point mutations and fusion transcripts) in BMSC of patients with the respective aberration in their leukemic clone. For this purpose we used both karyotyping (10 patients) and RT-PCR method. BMSC were analyzed by flow cytometry to evaluate the possible contamination with cells of hematopoietic lineage.

Results: We investigated the BMSC karyotype in seven patients and only one case led us to a remarkable finding. The clonal chromosomal rearrangement t(1;7) was detected in 25% of BMSC metaphases. Interestingly, this aberration was also detected in patient’s leukemic clone.

We also examined BMSC from leukemia patients bearing recurrent genetic abnormalities and in one case the leukemia-specific marker was detected by RT-PCR - we observed expression of ET6-V6RUNXI gene (0.02%) in BMSC by patient with (1;21) acute lymphoblastic leukemia. At the moment of BMSC culture initiation ET6-V6RUNXI expression in patient’s bone marrow was detected at high level [ET6-V6RUNXI/ABL=100:521%]. Before carrying out DNA extraction BMSC were harvested after the second passage and no contamination with CD45+/CD34+ cells by flow cytometry was observed (50,000 events collected from the sample). When BMSCs and Uke-1 cell line were cocultured by using 0.4 μm pore size both the BMSC population and K-562 were detected the Jak2V617F mutation (allele burden = 30,39%). We reproduced similar experiments with the K-562 cell line and got similar results - CD45+ cells were also detected in BMSC population (≈30%). Moreover we detected CD45+ non-cellular bodies using flow cytometry analysis. Implying K-562 cells are not likely to cross the semipermeable membrane (3,0 μm pores versus 20,0 μm cells as measured during microscopy). Besides BCR-ABL gene expression in BMSC was detected by RT-PCR (BCR-ABL/ABL=100:19%). We repeated same test with 0.4 μm pore inserts and without them in order to check implication of cell-to-cell interaction. We didn’t obtain any similar results with smaller pores, but the fusion transcript was detected in CD45+ BMSC population when these two cell populations weren’t devided. Both findings point out at possible horizontal gene transfer mediated by membrane vesicles larger than 0.4 μm and direct whole cell fusion.

Summary/Conclusions: Our data stands for the existence of horizontal gene transfer between leukemic clone and BMSC. This process seems to be mediated by membrane vesicles larger than 0.4 μm in size, though cell fusion can also take place. We also confirmed the fact BMSCs can bear clonal genetic rearrangements which are not specific to tumor cell populations. These findings show tight interaction between tumor and microenvironment cells and can partly explain nature of PCR-based MRD persistence in complete remission.

PB1854

CIRCULATING ENDOTHELIAL PROGENITOR CELLS AND METABOLIC FACTORS IN CHILDHOOD CANCER SURVIVORS

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Background: Circulating Endothelial Progenitor Cells (CEPCs) play a significant role in the maintenance of vascular integrity, balancing the co-agulation mechanisms and modulating the immune system by regulating the leukocyte trafficking, as well as controlling the vascular tone. Additionally, it is well-established, that patients who underwent chemotherapy have increased incidence of hypertension and obesity. Nevertheless, numerous studies have shown a negative correlation between CEPCs and obesity, underlining poor vascular repair.

Aims: The study of CEPCs in children who received chemotherapy for Acute Lymphoblastic Leukemia (ALL) and solid tumors (ST) and the investigation of their levels in correlation with patients Body Mass Index (BMI) and blood pressure (BP) regarding the time following treatment.

Methods: Circulating endothelial progenitor cells from children with ALL (n=77), ST (n=81) and children without malignancies as control group (n=71) were studied. Four colour flow cytometry was performed to determine the subpopulations CD34+CD45- and CD133+CD45-. CD34+ and CD133+ were detected using a PE-Cy7/EVGFIR2+ and CD133+ and CD45- in CD133+ and EVGFIR2+ of CEPCs. The BMI of the patients was calculated and the correlation of CEPCs and obesity underlining poor vascular repair was verified.

Results: Circulating Endothelial Progenitor Cells (CEPCs) play a significant role in the maintenance of vascular integrity, balancing the co-agulation mechanisms and modulating the immune system by regulating the leukocyte trafficking, as well as controlling the vascular tone. Additionally, it is well-established, that patients who underwent chemotherapy have increased incidence of hypertension and obesity. Nevertheless, numerous studies have shown a negative correlation between CEPCs and obesity, underlining poor vascular repair.
Results: The mean values of CEPCs subpopulation CD34+CD45negdimVEGFR2+ estimated in ALL, ST and Controls were 0.00386(SE=0.00072), 0.00613(SE=0.00146) and 0.002953(SE=0.0004) respectively. The mean percentage of CD34+CD45negdimCD133+VEGFR2+ in ALL, ST and Controls was 0.00331(SE=0.00072), 0.00499(SE=0.00113) and 0.002663(SE=0.00037). The correlation of CD34+CEPCs showed statistical significant difference of CD34+CD45negdimVEGFR2+ between the ST % BCP control 0.003174, 95CI of diff 7.716e-005 to 0.006272). In ALL the levels of CD34+CD45negdimVEGFR2+ the 1st year after treatment completion were 0.00458(SE=0.0026), during 1-3years 0.00311(SE=0.00066) and >3 years 0.003423(SE=0.00081). The levels of CD34+CD45negdimCD133+VEGFR2+ during the 1st year after chemotherapy was 0.0092(SE=0.0037), 1-3 years 0.0007(SE=0.00063) and >3 years 0.00303(SE=0.00081). The counts of CD34+CD45negdimVEGFR2+ in ST group the mean value of CD34+CD45negdimVEGFR2+ the 1st year after treatment was 0.0114(SE=0.0048),1-3 years 0.0047(SE=0.00133) and >3 years 0.0036(SE=0.0008). Whereas the percentage of CD34+CD45negdim CD133+VEGFR2+ the 1st year after chemotherapy was 0.0092(SE=0.0037), 1.3 years 0.0034(SE=0.00097)and>3 years 0.00336(SE=0.00085).Statistical significant results were calculated for the levels of CD34+CD45negdimVEGFR2+ in ST group between the groups <1 year and over years’ post treatment(Mean Diff 0.007747, 95 CI of diff 0.0002441 to 0.01525). The study of body weight in ALL and ST groups in relation with CEPCs showed no statistical significant difference, although a negative trend between obesity and CEPCs was found in the ALL group and a positive one in the ST group. The same trend also appeared in BP between ALL and ST regarding the CEPCs, with hypertensive patients in ALL group having higher levels of CEPCs than the ST hypertensive individuals.

Summary/Conclusions: The higher levels of CEPCs were estimated in ALL and ST just after treatment completion with a gradual decrease as time passes. The highest percentages of CEPCs were evaluated in ALL patients with normal weight and blood pressure in contrast with the solid tumor group. Further investigation is necessary to highlight the importance of these data.

PB1855

HEMATOLOGICAL PARAMETERS IN NATIVE HIGHLANDERS OF LADAKH AGED 4-19 YEARS

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Background: High altitude (HA) has always intrigued physiologists because of the remarkable ability of man to adapt to the hostile environment. Hematological changes associated with HA exposure is believed to be driven by hypobaric hypoxia of HA. Majority of the studies on HA physiology and hematological adaptation have focused on the hematological adaptation in lowlanders visiting HA or have compared the hematological profile of native highlanders from Andes and Tibet with those of the neighboring lowlanders. These studies have mostly been directed towards adult population with no or little reference to children and adolescent age groups. Moreover these studies have been done mostly on the highlanders of Andes and Tibet with no data on Indian highlanders.

Aims: We aimed at assessing hematological parameters in native highlanders in the age group of 4- 19 yrs and compare the same with Indian lowland population as well as native highlanders around the world. We have compared the hematological profile of Ladakh kids from the neighboring lowlanders.

Methods: A total of 390 native highlanders of Ladakh in the age group of 4-19 yrs with no history of travel to lowland were taken for the study. A written informed consent was taken from the parents of all the subjects before starting interviewing them for the laboratory investigations. After taking antiseptic precautions, blood samples were drawn from the antecubital vein and complete hemogram including red blood cell indices were measured. The study subjects were stratified into five age groups (less than 5y, 5-8y, 8-10y, 10-12y, 12-15y and children more than 15y). Appropriate statistical analysis was done to compare the hematological parameters between the stratified age groups as well as between boys and girls.

Results: A total of 197 girls and 193 boys were included in the study. The mean age of the subjects was 128±80 (means±SD) months. The mean hematocrit value increased with age (38.68±2.51% in <5 yrs age group to 43.84±2.04% in >15 yrs age group). Similarly the mean corpuscular volume (MCV) also showed a rising trend with age (79.07±3.36 fL in <5 yrs age to 43.84±2.04% in >15 yrs age group). Similarly the mean corpuscular volume to crit value increased with age (38.68±2.51% in <5 yrs age group to 43.84±2.04% in >15 yrs age group). Similarly the mean corpuscular haemoglobin concentration (MCHC) decreased with age from 36.91±2.85% at <5 yrs of age to 35.69±0.94%. The mean platelet count in boys was significantly higher than in girls (p=0.0003) (Figure 1).

Figure 1.

Summary/Conclusions: The hematological adaptation of Ladakhi kids is different as compared to other native highlanders. There is also a significant difference in the hematological response to hypobaric hypoxia with growing age and between boys and girls.

PB1856

AGE VARIATION OF B-CELL PRECURSORS IN BONE MARROW: NORMAL VALUES AS A REFERENCE FOR MDS IN BRAZIL


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Background: Decrease of bone marrow (BM) B-cell precursors (BCP) is an important diagnostic feature in myelodysplastic syndromes (MDS). Moreover, their number is associated with patients’ overall survival. However, BCPs vary with age in normal BM.

Aims: In a multicenter study from the Brazilian Group of Flow Cytometry we analyzed the variation of BCPs in normal BM according to age, antibody combinations used for quantification and reproducibility after a centralized reanalysis. We set up a reference pattern of normal values for evaluation of patients with a suspected MDS.

Methods: In a retrospective study including 10 centers we retrieved analyses of BM donors and cases examined for elucidation of transitory reactive cytopneas presenting a normal BM immunophenotyping. BCPs were enumerated as CD19/CD34/CD45/CD10 cells (panel 1) or CD19/CD34/CD45 cells (panel 2), among the total nucleated cells and as percentage among CD34+ cells. Standardisation: multiple regression to analyse the dependence of BCS from the variables analysed.

Results: 134 cases were included. Panel 1 was applied in 106 cases (all centers) and panel 2 was used in 28 cases (3 centers). Age range: 10 months to 89 years. In the same age range, values for panel 2 were lower than those for panel 1, in multiple regression % BCP total cells—0.313 (for panel 2)+correction factor for labs +1.873. The correction factor for labs was 0 to -0.40. Age explained alone 49.6% of the variance of BCPs/total cells, while “laboratory” explained 5.2% and panel used explained only 0.8%. Age explained only 24.9% of the variance of BCPs/CD34+ cells.

Table 1.

Summary/Conclusions: In a normal population BM B-cell precursors varied mainly with age, but were also dependent on technical peculiarities of operators and equipments. Analysis by phenotype and as percentage of total cells was more accurate and less susceptible to variation.
Background: Disrupted hematopoiesis is life-threatening complication of allo- geneic hematopoietic cell transplantation (allo-HCT). The interactions of haematopoietic stem/progenitor cells (HSPCs) and bone marrow (BM) microenvironment, niche(s), control the homeostasis of BM. TGF-b induced gene 3 (BIG3), one of BM extracellular matrix (ECM) which is produced by niche cells maintain the homeostasis and regeneration of BM.

Aims: We analyzed the relationship between the idiopathic thrombocytopenia after allo-HCT and the BM expression of periostin as the only paralogue of BIG3.

Methods: We reviewed twenty patients who transplanted with matched sibling donor for acute myelogenous leukemia at Kyungpook National University Hospital from January 2010 to August 2015. BM biopsy specimens at the time of day 28, day 90, day 180, and day 365 since allo-HCT were decalcified and stained with primary antibody of BIG3 and periostin. Expression of periostin in BM slides were reviewed by pathologist as follows: normal (0), minimal staining around blood vessels; (+1), sparse staining and/or focally staining; (+3), diffuse and strong staining; (+2), between (0) and (+3).

Results: The median age at transplant was 38.5 years (range, 17-68 years) and male was 13 patients (65%). Twelve patients (60%) were in CR1 (complete remission), 8% in CR2. Thirteen patients (65%) received myeloablative conditioning regimen. The median dose of CD34+ cell was 3.67×10^6/kg (range, 1.6-7.67×10^6/kg). All patients achieved the neutrophil engraftment with a median time of 13 days (range 9-24days). The median time of platelet engraftment was 15.5 days (range, 13-77days). Idiopathic thrombocytopenia developed as follows; 13 patients at day 28, 16 at day 90, 6 at day 180, and 3 at day 365. There was no significant difference between idiopathic thrombocytopenia and the expression of BIG3 or Periostin (p=0.128). However, BM idiopathic thrombocytopenia manifested the low periostin/BIG3 ratio (p=0.007). Acute GVHD was observed in 12 patients (60%) and chronic GVHD developed in 13 patients (65%). The development of thrombocytopenia dose not differ according to acute and chronic GVHD (p=0.847) (Figure 1).

Summary/Conclusions: The periostin/BIG3 ratio might represent the status of BM niche during the homeostasis and regeneration of hematopoiesis. High periostin/BIG3 ratio could predict the recovery of the idiopathic thrombocytopenia.

PB1858

ASSOCIATION WITH OMENN SYNDROME AND CYSTINURIA: CASE REPORT

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Background: Omenn syndrome is one type of combined immunodeficiency, characterized with hematopoesismegaly, lymphadenopathy, recurrent infections and has an autosomal recessive pattern of inheritance. T lymphocyte count cannot be easily recognized by their similarity with MGG images. Erythrocytes could be easily recognized by their similarity with MGG images. Erythrocytes exhibited the shortest lifetimes (210.4±42.1 ps). Normal shaped erythrocytes in smears of sickle cell patients showed similar values (214.6±3.1 ps), whereas crenated erythrocytes as well as drepanocytes revealed significantly elevated values (314.2±66.7 ps and 312.5±67.0 ps respectively). Regarding erythropoiesis, the cytoplasm of erythroblasts showed significantly shorter lifetimes (623.5±272.1 ps) than that of myeloblasts (835.9±198.4 ps) and the same was the case when comparing the nuclei (erythroblasts: 895.4±262.8 versus myeloblasts: 1166.4±287.9 ps). The same differences could be found in megaloblastic anemia. There were no significant differences between the FLIM values of the different cell types between normal hemopoiesis and megaloblastic anemia.

Summary/Conclusions: The FLIM technique is easily applicable on unstained routine smears and revealed images of good quality permitting cell identification. It allowed also to distinguish between erythroid and myeloid precursors cells and indicates the major physico-chemical changes during the process of falcization.
on Table 1. There are three deaths because of refractory diseases. Five patients needed treatment for the first disease and nine patients needed treatment for the second disease. Four patients had treatment for both diseases.

Table 1.

<table>
<thead>
<tr>
<th>#</th>
<th>Gender</th>
<th>Age</th>
<th>Stage</th>
<th>Treatment</th>
<th>ESR</th>
<th>Stage</th>
<th>Treatment</th>
<th>Karyotype</th>
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<tr>
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<td>67</td>
<td>I</td>
<td>ABVDx6</td>
<td>5</td>
<td>II</td>
<td>ABVDx5</td>
<td>BCR-ABL</td>
<td>6</td>
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<tr>
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<td>F</td>
<td>78</td>
<td>II</td>
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<td>7</td>
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<td>ABVDx8</td>
<td>BCR-ABL</td>
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</tr>
<tr>
<td>3</td>
<td>M</td>
<td>68</td>
<td>III</td>
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</tbody>
</table>


Summary/Conclusions: occurrence of two malignancies in the same patient can be a challenge for the hematologist. Findings of the second disease can be attributed to the first disease or considering them to be results of treatment. Follow up and initiation of treatment in those patients can be more complex than usual. As far as origin is concerned there are conflicting reports in the literature supporting a common or different cells of origin. Recording of these cases and biobanking can be of great interest for understanding mechanisms of hematologic neoplasms.

PB1861

B SYMPTOMS AND ELEVATED ESR AS PREDICTORS OF OVERALL SURVIVAL IN HODGKIN LYMPHOMA. A 20 YEAR FOLLOW UP MULTICENTER ANALYSIS.

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Background: The prognosis of Hodgkin lymphoma (HL) has improved significantly with the implementation of a risk-adapted treatment that combines chemo and radiotherapy. Although this approach has led to the greatest advance in disease response, the benefit in terms of overall survival (OS) has been jeopardized by long term toxicity.

The identification of risk factors is crucial to assign each patient to a well defined risk group and prevent under or overtreatment, minimizing the risk of relapse and long term toxicity.

Aims: To analyze the risk factors associated with survival in HL treated with an ABVD based regimen that restricted radiotherapy only to bulky disease.

Methods: We retrospectively analyzed HL patients diagnosed in 4 centers in Tarragona area (Catalonia, Spain), between 1995 and 2015, treated uniformly according to a local protocol.

Patients were assigned into 4 groups: G1: favorable early stage: ABVDx6 cycles, G2: bulky early stage without other risk factors: ABVDx6+IFRDT. G3: unfavorable early stage (B symptoms) and advanced stage without bulky disease: ABVDx8, G4: Bulky advanced stage: AVBDx8+IFRDT

Results: A total of 183 patients were analyzed with a median follow up of 82 months [range 1-244]. Male/female ratio was 1.29. Median age was 36 years [range 16-82]. Complete response was achieved in 160 patients (87.4%). The estimated OS at 20 years for the whole group was 62.7%. Kaplan–Meier method and log rank test were used for survival analysis. Cox proportional hazards model was used for univariate analysis to identify predictive factors for OS. Factors with significance (p<0.05) were considered for multivariate Cox regression. In univariate analysis, worse OS was found in patients with increased LDH, non-NS subtype, albumin <3.5 g/dL, B symptoms, HIV+, advance stage and ESR >50 mm (log rank: p=0.012; p=0.049; p=0.024; p=0.002; p=0.005; p=0.004 and p=0.001 respectively). The multivariate Cox regression analysis identified B symptoms and ESR >50 mm as independent prognostic factors for OS (p=0.002; p=0.006 respectively). These variables allowed us to identify 3 patient groups: low (no risk factors), intermediate (either B symptoms or ESR>50 mm) and high risk (both risk factors), with significant differences in OS. Estimation for OS was uniformly analyzed at 216 months (18 years), which is the shortest follow up period for patients in the low risk group. Patients in the low, intermediate and high risk groups had an estimated OS of 85.7%, 65% and 40.1% (p<0.001) (Figure 1).

Figure 1.

Summary/Conclusions: B symptoms and ESR>50mm are independently associated with OS. The combination of these factors can stratify patients in low, intermediate and high risk groups with significant differences in OS, regardless their clinical stage.
ADVANCED HODGKIN LYMPHOMA PATIENTS WITHOUT LARGE TUMOR MASS – A NEW PROGNOSTIC SCORE IDENTIFIES PATIENTS WITH FAVORABLE OUTCOME

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Background: ABVD and escalated BEACOPP are still the standard of care in patients with advanced Hodgkin Lymphoma (HL). The use of escalated BEACOPP gives better disease control but it is associated with more acute and late toxic effects. The identification of patients who require more or less aggressive initial approach remains the main goal for many investigators in the field of HL.

Aims: The aim of this study was to identify among patients with diagnosed advanced HL without large tumor mass the subgroup which should not be considered for more aggressive approach than ABVD.

Methods: A retrospective study was performed on 149 patients classical HL, diagnosed in the period June 1997-December 2011. All the patients were in clinical stage III or IV and didn’t have any tumor lesion of 5 cm or more in its longest diameter. The standard of initial care was 6-8 cycles of ABVD followed by radiotherapy. Prognostic relevance of age more than 45 years, gender, CS IV, presence of B symptoms, IPS score, ESR≤50 mm/h, Hgb<10.5 g/dL, WBC≤15,000 mm³ and lymphopenia (lymphocytes <600/mm³ or <8% of WBC count) were examined.

Results: The median age of analysed patients was 37 (range 17-80). The median follow up was 98 months. For the whole group 5-year event free survival (EFS) was 63.1% and 5-year overall survival (OS) was 80.6%. In univariate analysis, worse OS was found in patients older than 45 years (5-year OS 66.7% vs 87.8%), patients with CS IV (5-year OS 70.2% vs 87.0%), B symptoms (5-year OS 77.1% vs 89.1%), ESR>50 mm/h (5-year OS 75.0% vs 89.5%), lymphopenia (5-year OS 65.6% vs 84.6%) (log rank; p=0.001, p=0.006, p=0.040, p=0.003, p=0.010, respectively), while gender, anaemia and leukocytosis didn’t influence OS (log rank; p=0.303, p=0.714, p=0.522, respectively).

Worse EFS was found in patients with CS IV (5-year EFS 50.0% vs 70.7%, kog rank p=0.002), IPS≥3 (5-year EFS 53.8% vs 73.2%, (log rank; p=0.006) and lymphopenia (5-year EFS 50.0% vs 66.7%, kog rank p=0.025), while age, gender, B symptoms, ESR>50 mm/h, anemia and leukocytosis didn’t influence EFS (log rank; p=0.078, p=0.437, p=0.068, p=0.151, p=0.384, p=0.158, respectively). The multivariate Cox regression analysis showed that identified age more than 45 years, ESR>50 mm/h and lymphopenia as independent prognostic factors for OS, while only IPS was identified as an independent factor for EFS. Afterwards, we performed survival analysis with aggregate scores of identified negative prognostic factors for OS for each patient. Since there was no difference in OS in intergroup analysis, groups with similar risk of survival were formed. By applying PET/CT results two pts’ groups were formed: 1.group (n=73 pts) with PET/CT positive results (partial metabolic response or progressive disease). Using Deauville criteria complete response was observed in 95 (70.7%) NHL patients and 71 (77.6%) HD pts. 2.group (n=153 pts) with negative PET/CT results (Deauville score 1-3) and 2.group (n=153 pts) with negative PET/CT results (Deauville score 4-5) were detected in 39 (29.1%) and 34 (37%) NHL and HD pts, respectively. 22 nd Congress of the European Hematology Association

Treatment Escalation in Case of Positive PET 2 and Impact of Early PET in Extensive Stage Hodgkin Lymphoma

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Background: ABVD therapy has been for a long time the reference to standard chemotherapy not only for determination of those who need additional intensive treatments. The aim of our study is also to identify the higher risk patients for whom more intensive treatment could be used as first-line treatment.

Aims: The aim of the study was to assess the clinical value of 18F-FDG PET/CT in lymphoma pts with Hodgkin’s disease (HD) and non-Hodgkin’s lymphoma (NHL).

Methods: Two hundred and twenty six pts with biopsy proven lymphoma – (HD n=92 and NHL n=134), aged 18-76, were retrospectively reviewed. These pts were examined 4-6 weeks after the completion of the standard chemotherapy which pts would benefit from additional treatment. PET/CT was used to assess response in FDG-avid histologies using 5-point scale, both for interim analysis and treatment end assessment. The Lugano classification has proved extremely useful in the standardization of treatment response. A score 1, 2, 3 is considered to represent complete metabolic response; score of 4, 5 – partial, no response or progressive disease.

Results: By applying PET/CT results two pts’ groups were formed: 1.group (n=153 pts) with negative PET/CT results (Deauville score 1-3) and 2.group (n=73 pts) with PET/CT positive results (partial metabolic response or progressive disease). Using Deauville criteria complete response was observed in 95 (70.7%) NHL and 58 (63%) HD pts. These pts were in continuous complete remission. Partial response, stable or progressive disease (Deauville score 4-5) were detected in 39 (29.1%) and 34 (37%) NHL and HD pts, respectively. One hypermetabolic lesions and disseminated nodal or extranodal involvement were detected in 15 and 24 NHL pts as well in 12 and 22 HD pts. Two hundred and two hypermetabolic lesions were considered for radiotherapy, while pts with more than one nodal or extranodal lesions after completion of standard chemotherapy were considered for high dose chemotherapyautologous stem cell transplantation (ASCT).

Summary/Conclusions: 18F-FDG PET was useful in HD and NHL pts after standard chemotherapy not only for determination of those who need additional therapy, but for the choice of the further management: radiotherapy, chemotherapy, or ASCT. A negative PET/CT study after the completion of therapy is an excellent predictor of good prognosis.

PB1865

THE PROGNOSTIC IMPACT OF 18F-FDG PET/CT IN LYMPHOMA PATIENTS AFTER STANDARD CHEMOTHERAPY

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Background: The lymphomas are a heterogeneous group of malignant diseases. The exact diagnosis, precise staging and follow up is very important for treatment and prognosis of these patients (pts). Accurate pretreatment evaluation and response assessment are critical to the optimal management of lymphoma pts. Differentiation of post-therapeutic residual tissue from active lymphoma is unsatisfactory when using only morphological imaging approaches. Positron emission tomography/computed tomography (PET/CT) is the most sensitive and specific imaging technique for monitoring therapy response currently available for lymphoma pts after standard chemotherapy and determining which pts would benefit from additional treatment.

Aims: The aim of the study was to assess the clinical value of 18F-FDG PET/CT for staging and disease evaluation in lymphoma pts with Hodgkin’s disease (HD) and non-Hodgkin’s lymphoma (NHL).

Methods: Two hundred and sixty six pts with biopsy proven – lymphoma pts after standard chemotherapy and determining which pts would benefit from additional treatment.

Results: By applying PET/CT results two pts’ groups were formed: 1.group (n=153 pts) with negative PET/CT results (Deauville score 1-3) and 2.group (n=73 pts) with PET/CT positive results (partial metabolic response or progressive disease). Using Deauville criteria complete response was observed in 95 (70.7%) NHL and 58 (63%) HD pts. These pts were in continuous complete remission. Partial response, stable or progressive disease (Deauville score 4-5) were detected in 39 (29.1%) and 34 (37%) NHL and HD pts, respectively. One hypermetabolic lesions and disseminated nodal or extranodal involvement were detected in 15 and 24 NHL pts as well in 12 and 22 HD pts. Two hundred and two hypermetabolic lesions were considered for radiotherapy, while pts with more than one nodal or extranodal lesions after completion of standard chemotherapy were considered for high dose chemotherapyautologous stem cell transplantation (ASCT).

Summary/Conclusions: 18F-FDG PET was useful in HD and NHL pts after standard chemotherapy not only for determination of those who need additional therapy, but for the choice of the further management: radiotherapy, chemotherapy, or ASCT. A negative PET/CT study after the completion of therapy is an excellent predictor of good prognosis.

PB1865

BCL-2 AND CD30 EXPRESSION IN HODGKIN AND REED-STERNBERG CELLS OF CLASSICAL HODGKIN’S LYMPHOMA AS A POORER PROGNOSIS CRITERIA

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Background: There are a lot of prognosis criteria for risk stratification of Hodgkin’s lymphoma. (HL). The most applicable is the IPS-7, however this score is ignoring a tumor cells phenotype. There are data about dependence survival and antigenic profile of Reed-Sternberg (RS) cells. To determine the clinical significance of bcl-2 and CD30 expression in RS cells of classical HL, we have correlated its expression with available IPS criteria and failure-free survival (FFS).

Aims: To determine predictive possibility proapoptotic protein bcl-2 and CD30 antigen on RS cells aggregating with criteria IPS.
Methods: In study were included 85 previously untreated patients, presented with classical HL between 2002 and January 2016. This retrospective study did not require approval by the Local ethical committee. Inclusion criteria were: a histologically confirmed diagnosis of classical HL, the presence of a fixed in paraffin before treatment a lymph node sample or other diseased tissue, the median follow-up was not less than 18 months.

Results: In the study population (n=85) identified 30 (35%) histological samples bcl-2+, and 55 biopsies (65%), bcl-2. Group bcl-2+ patients had a lower response rate after ABVD chemotherapy - only 24 (28%) patients achieved CR or better result, as compared with 49 patients (57.6%) of the bcl-2 group. Three-year event-free survival (EFS) in bcl-2+ patients had lower 82% vs 96% in bcl-2 group (p=0.018). Multivariate analysis using the Cox proportional-hazard model with the inclusion of bcl-2+, CD30 +; bcl-2+/CD30 +, age 45 and older. B-symptoms, III-IV stage, anemia, decreased serum albumin, increased LDH, leukocytosis revealed that the expression of bcl-2 on RSs was an independent factor of poor prognosis. 3 year EFS was 52% vs 90% in bcl-2 population (p=0.022; RR=1.4). The greater relative risk was observed in a population with double expression of bcl-2 and CD30, where the 3-year EFS was 47% (p=0.012; RR=1.6).

Summary/Conclusions: The expression of bcl-2 on HRS cells can be an independent prognostic factor, co-expression of bcl-2 and CD30 can be viewed as a more powerful factor of poor prognosis than bcl-2+ cells.

PB1866

SURVIVAL ANALYSIS OF PATIENTS WITH CLASSICAL HODGKIN’S LYMPHOMA TREATED WITH ABVD: RESULTS FROM TWO REFERRAL CENTERS IN MEXICO CITY.

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Background: Classical Hodgkin’s lymphoma (cHL) is a neoplastic disease with a favorable prognosis since 85% of patients can be considered cured with current treatment strategies. Combined chemotherapy with Adriamycin, Bleomycin, Vinblastine and Dacarbazine (ABVD) has been the standard therapy for over 20 years. Epidemiological information and the regimen’s results as first-line therapy in Mexico are limited.

Aims: The aim of this study was to conduct a survival analysis in adult patients from two referral centers in Mexico City.

Methods: This is a retrospective analysis of all patients with cHL treated at the Instituto Nacional de Cancerología and the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, between 2009 and 2013. The study was approved by the local Ethics Committee.

Results: We included a total of 193 patients with a de novo diagnosis and initially treated with ABVD: 60.6% of cases were male, with a median age of 36 years (17-81 years), 71.5% were diagnosed in late clinical stages (CS). The most frequent histopathological subtypes were: nodular sclerosis and mixed cellularity (46.6% and 40.9%; respectively), of the observed 90% response rate (RR) was 85.7% [Complete response (CR) was 78.2%]. The RR was 90% in early CS vs 83.8% in late CS (CR rate was 84% vs 75.8%; respectively, p=0.23). Univariate analysis by logistic regression in the early CS group revealed that having a Lymphocyte:Monocyte ratio <1 presents an unfavorable tendency to achieve CR [OR 0.150 (95%CI 0.018-1.274; p=0.082)]. In the group in late CS, we found that the lymphocyte percentage tended to favor CR [OR 1.048 (95%CI 0.994-1.015; p=0.081)] and the opposite was observed in terms of the absolute monocyte count [OR 0.999 (95%CI 0.998-1.000; p=0.082)]. Median follow-up was 35 months (0-96 months), 10.9% of cases had died at last follow-up, and median overall survival (OS) of the entire cohort had not been reached at the time of analysis (5-year OS, 87.1%). However, at the time of this analysis, the group of patients in complete remission had a greater OS than the group that did not achieve CR (p=0.0001). With Cox multivariate analysis of OS according to CS, we detected that in the group in early CS, none of the analyzed factors were significant, while in the late CS group, age >45 years was an independent risk factor [HR 6.9 (95%CI 1.80-26.60; p=0.005)] and achieving CR had a protective effect [HR 0.02 (95%CI 0.004-0.108; p=0.0001)].

Summary/Conclusions: Although OS medians had not been reached at the time of analysis, it is noteworthy that CR (84%) in early CS is lower than that reported in the literature and no related prognostic factor has been identified. The role of lymphocytes and monocytes may prove to be significant in larger series with a longer follow-up.

PB1867

OUTCOME OF PD-1 BLOCKADE IN PATIENTS WITH RELAPSED HODGKIN LYMPHOMA AND ACTIVE GRAFT-VERSUS-HOST DISEASE

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Background: Efficacy of PD-1 (programmed death-1) inhibitors in relapsed/refractory Hodgkin lymphoma (HL) has been established, but their role in relapse after allogeneic stem cell transplant (alloSCT) remains controversial due to the perceived risk of exacerbating graft-versus-host disease (GVHD). The literature is largely limited to case reports in patients with no or quiescent GVHD.

Aims: To determine the outcome of PD-1 inhibitor therapy and subsequent management in patients with concomitant biopsy proven active GVHD and progressive HL after alloSCT.

Methods: We describe the treatment and management of two patients in our centre.

Results: Case 1 had both extensive bony, lung and nodal HL with active skin, pleuroporcardiac and liver GVHD 6 months after donor leucocyte infusion (DLI) and immunosuppression withdrawal and 24 months after sibling alloSCT. Fifty-five of the standard pembrolizumab dose (100mg) produced a PET partial response after 5 weeks but with concomitant biopsy proven, severe exacerbation of liver GVHD. The latter was managed with prednisolone, everolimus, ursodeoxycholic acid (UDCA) and subsequently tacrolimus with gradual but substantial improvement in liver function over the next 5 months (Figure 1) in the absence of further PD-1 blockade, but with progression of lymphoma. Pembrolizumab 50mg was then given with lymphoma response but again a significant (but less severe) flare of liver GVHD occurred. Subsequent 25mg doses failed to prevent lymphoma progression. Reintroduction of 50mg doses approximately each 6 weeks for 4 doses with prophylactic everolimus, low dose prednisolone and ruxolitinib, has resulted in ongoing substantial but incomplete PET responses with associated stable liver GVHD. Case 2 had progressive mediastinal and pulmonary HL despite DLI-induced extensive liver and skin chronic GVHD 38 months post sibling alloSCT. Initial therapy consisted of optimisation of liver GVHD with 8 weeks of UDCA and prednisolone with improvement in liver indices (Figure 1). Pembrolizumab 50mg was then given, together with sirolimus and ruxolitinib as GVHD ‘prophylaxis’, resulting 5 weeks later in complete metabolic remission on PET. Concomitantly liver GVHD was aggravated (See Figure 1) together with pancytopenia and narrow hypoplasia attributed to an immune-mediated phenomenon. Despite addition of tacrolimus and increased steroids, he remains with severe liver dysfunction and pancytopenia 10 weeks after the single dose of PD1 inhibitor therapy.

Summary/Conclusions: PD-1 inhibitors can exert powerful graft vs HL effects even in patients with progression in the context of active GVHD, but at the expense of substantial GVHD exacerbation. Further exploration of approaches such as individualised dose titration according to response and cell activity and prophylactic therapy with non-calcineurin based immunosuppression which may not mitigate the anti-lymphoma effect will help evaluate whether durable responses with tolerable toxicity is possible in this context.

Figure 1.

Summary/Conclusions: PD-1 inhibitors can exert powerful graft vs HL effects even in patients with progression in the context of active GVHD, but at the expense of substantial GVHD exacerbation. Further exploration of approaches such as individualised dose titration according to response and cell activity and prophylactic therapy with non-calcineurin based immunosuppression which may not mitigate the anti-lymphoma effect will help evaluate whether durable responses with tolerable toxicity is possible in this context.
PB1868
PROGNOSTIC VALUE OF THE RED CELL DISTRIBUTION WIDTH IN PATIENTS WITH CLASSIC HODGKIN LYMPHOMA
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Background: The current gold standard for risk stratification in Hodgkin lymphoma (HL) is the International Prognostic Score. There are certain molecular and immunohistochemical prognostic markers in patients with HL, but their cost and technical constraints make such an application in routine impractical and expensive. Therefore, prognostic models for classic HL (cHL) that are inexpensive, simple, and easy to perform and interpret are needed.
Methods: We retrospectively analyzed data from 54 cHL patients diagnosed from 2005 to 2016 at the University Hospital Center Osijek, Osijek, Croatia. We evaluated disease outcome, overall survival (OS) and event-free survival (EFS), and demographic, clinical and laboratory factors affecting outcome. Univariate analysis and Cox regression analysis were used.
Results: The median age of patients was 36 years, 29 men (54%). Higher RDW levels (%) were found in patients with advanced Ann Arbor clinical stage (15.34 ± 2.28 vs 13.12 ± 1.3, P < 0.001) and in those with poor response to therapy (15.65 ± 3.37 (progression) vs 16.68 ± 2.09 (partial remission), 13.95 ± 1.82 (complete remission), P = 0.008). Patients with RDW values of >14.5% (cutoff value calculated by receiver-operating characteristic) had a significantly worse two-year EFS (62.4% vs 90.4%, P = 0.009) but did not differ significantly in terms of OS (P = 0.2). Univariate analysis revealed that a high RDW (>14.5%) was correlated with poor EFS (P = 0.019). Multivariate Cox regression analysis showed that RDW >14.5% was an independent prognostic factor for EFS (hazard ratio [HR] 3.801, 95% confidence interval [CI] 1-14.45, P = 0.05). The RDW allowed further borderline statistically significant risk stratification in patients who were considered to be at low risk on the basis of an International Prognostic Score less than 4 (P = 0.053).
Summary/Conclusions: High baseline RDW is an independent prognostic marker of poor outcome in patients with cHL. RDW ratio is simple, inexpensive, and independent prognostic factor for EFS that may improve the ability to identify high-risk patients with cHL. It could be an easily available and inexpensive marker for the risk stratification in patients with cHL.

PB1869
HIGH FREQUENCY OF SECONDARY MALIGNANCIES IN PATIENTS WITH LARGE GRANULAR LYMPHOCYTIC LEUKEMIA: A SINGLE INSTITUTIONAL EXPERIENCE
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Background: Large granular lymphocyte (LGL) disorders represent a spectrum of aberrant T-cell or natural killer cell lymphocytic proliferations. LGLL is classically associated with autoimmune conditions and bone marrow (BM) failure disorders. SM has been reported in association with LGLL in about 10%. Aims: The aim of this study is to evaluate the impact of SM on the clinical course of LGLL.
Methods: This is a retrospective study of LGLL patients evaluated at Moffitt Cancer Center between January 1995 and May 2016. The diagnostic clinicopathological criteria consisted of LGL count > 0.5 k/µl with T-cell receptor gene rearrangement. Lower absolute number of clonal circulating LGLs with characteristic immunophenotype associated with BM involvement, cytopenias, myelodysplasia and/or associated symptoms were also diagnostic. Patients with myelodysplastic syndrome were excluded. Survival analysis was performed using the Kaplan-Meier method with log-rank test. Chi-square and T-test were used to analyze association among various variables. Significant P-value was considered < 0.05.
Results: Out of 668 screened patients with LGL expansions in peripheral blood, 261 met criteria for LGLL. Secondary malignancies were present in 44% (116/261) of LGLL patients, of which 38% were hematological and 80% arose prior to onset of LGLL. Most common solid secondary malignancy included skin cancer (14%), prostate cancer (12%), and breast cancer (12%), while most common hematological secondary malignancy consisted of non-Hodgkin lymphoma (17%) and chronic leukemia (14%). 5-year overall survival (OS) for all LGLL patients was 75% and 10-year OS 63%. There was a statistically significant difference in 5-year OS between LGLL patients with a secondary malignancy compared to without (p = 0.049), but no difference between both groups in median OS or 10-year OS. Patients diagnosed with a secondary malignancy prior to LGLL had worse 5-year OS (p = 0.031) and 10-year OS (p = 0.05) compared to all other LGLL patients.
Summary/Conclusions: This study showed that the frequency of a secondary malignancy is higher than previously described, especially with onset prior to diagnosis of LGLL. Even though median age of LGLL is around 60 years, it appears that age itself cannot explain this phenomenon. Our results suggest that having a secondary malignancy is a poor prognostic factor in LGLL patients.

PB1870
BENDAMUSTINE AND RITUXIMAB COMBINATION THERAPY WITH SUBSEQUENT RITUXIMAB SUPPORTING THERAPY IN RUSSIAN SUBJECTS WITH RELAPSED OR REFRACTORY INDOLENT B-CELL NON-HODGKIN LYMPHOMAS
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Background: Combination of bendamustine and rituximab has been established in many international guidelines as treatment for patients with indolent B-cell non-Hodgkin lymphoma (iNHL).
Aims: The objective of this study was to evaluate the effectiveness, safety, and tolerability of bendamustine/rituximab combination followed by rituximab maintenance therapy for relapsed or refractory (R/R) iNHL patients in the Russian Federation.
Methods: Adult subjects (≥18 yr), diagnosed with R/R iNHL according to local diagnostic standards, and were enrolled in this prospective observational study. Intravenous therapy was administered in 2 stages (Figure 1): a combination therapy stage followed by a rituximab supporting therapy stage for subjects who achieved complete response (CR) or partial response (PR) during the combination therapy stage. Overall response rate (ORR) was assessed after
3 (Evaluation 1) and 6–8 (Evaluation 2) 28-day cycles. Data from the full analysis set (FAS) were used for the primary analysis and the per-protocol (PP) set for a subgroup analysis. Safety/tolerability was a secondary endpoint and was assessed in the safety analysis set (SAF). Response assessments used the LOCF method for substitution of missing data; overall survival (OS) and progression-free survival (PFS) were calculated using Kaplan–Meier estimates, safety/tolerability was assessed by adverse event (AE) frequency and described using descriptive statistics.

Results: Of the 102 subjects enrolled between June 2012 and October 2015, 83 subjects (52M/31F; median age 59 yr [range: 27–84]) with various NHL histology; subjects with mantle cell lymphoma [n=4], diffuse large B-cell lymphoma [n=2], and follicular lymphoma transformation [n=1] were excluded from the PP population due to deviation from the NHL inclusion criteria. Most study subjects were heavily pretreated with a median number of 2 prior lines of therapy before entering the study (range: 1–6). At Evaluation 2, ORR in the FAS was high (n=32/63 [51.0%]) with 35 (54.2%) subjects achieving CR (confirmed, n=20 [31.7%]; unconfirmed, n=15 [18.1%]) and 23 (37.7%) achieving PR; ORR (defined as [CR+CR unconfirmed +PR]) in the PP population was 70.8% (Table 1). For FAS patients, at follow up (17 mo) neither median OS nor PFS had been reached; 2-year OS was 88.9% (95% CI: 79.7–98.0%) and 2-year PFS was 87.9% (95% CI: 80.7–95.7%). In the SAF, 31 of 96 subjects (32.3%) reported ≥1 AE. Decreased neutrophil count, decreased white blood cell count, and infections were the most commonly reported AEs and serious AEs. Twelve deaths occurred: 5 due to disease progression (n=2) or relapse (n=3), 5 were not related to lymphoma or occurred during remission, 1 cause of death was unknown, and 1 subject died from hyperthermia and respiratory failure, which was the only death in the study considered related to combination therapy.

Figure 1.

Summary/Conclusions: Bendamustine plus rituximab therapy followed by rituximab maintenance therapy was generally well tolerated and demonstrated clinical effectiveness in Russian R/R patients with INHLs. Although a number of subjects with aggressive lymphomas were included in the FAS, the ORR rate was not considerably different from the PP population (ORR: 69.9% [FAS] vs 70.8% [PP]).

PB1871

PROGNOSTIC VALUE OF G8 SCREENING TOOL IN PATIENTS WITH INDOLENT B-CELL LYMPHOPROLIFERATIVE NEOPLASMS – A SINGLE CENTRE EXPERIENCE

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Background: Indolent B-cell lymphoproliferative neoplasms (B-LPN) are malignant diseases of advanced age. The most common among them, follicular lymphoma (FL), marginal zone lymphoma (MZL) and chronic lymphocytic leukemia (CLL) together represent about 40% of all B-LPN. However, as indolent B-LPN are most often the slow-growing diseases, an approach "watch and wait" is often recommended. But, when treatment is necessary, the advanced patients' age indicate the need for geriatric assessment (GA) in aim to indentify functional, cognitive, social, nutritional and psychological parameters of the general health status. G8 screening tool (G8) is recognized as fast, easy to perform and sensitive test for selecting the group of fit elderly patients capable for curative treatment approach. Although incorporated in routine oncological practice, GA and G8 are not widely evaluated in hematological practice, so far.

Methods: Total of 89 consecutive elderly patients (45males and 44 females with median age at diagnosis 74.5 years, range 46–86 years were included). Seventeen were indolent B-LPN (24 with FL, 26 with MZL and 39 with CLL) who fulfilled criteria for treatment initiation were included in study. Patients were treated with antracycline, fluorouracil, or alkylated agents based chemotherapy regimens +/- monoclonal anti-CD20 antibody. Validity of G8 was compared with standard relevant clinical indices of fit elderly patients capable for curative treatment approach. Although incorpo-rated in routine oncological practice, GA and G8 are not widely evaluated in hematological practice, so far.

Results: Of all 89 patients median overall survival (OS) was 77 months, and disease free survival (DFS) in 58 (77.3%) patients achieving remission was 25 months. Among laboratory parameters, hemoglobin, platelet, neutrophil and monocyte count, as well as C-reactive protein, beta-2 microglobulin didn't influence OS and DFS. Elevated lactate dehydrogenase was found significant for CR rate, and low albumin level (<40g/L) for predicting OS. Among clinical parameters age, sex, presence of "B" symptoms, splenomegaly (>13cm), bulky disease (>10cm), extranodal (EN) disease, as well Charlson comorbidity index (CCI: ≥3) and ECOG performance status (PS; ≥2) and G8 screening tool (G8) are recognized as fast, easy to perform and sensitive test for selecting the group of fit elderly patients capable for curative treatment approach. Although incorporated in routine oncological practice, GA and G8 are not widely evaluated in hematological practice, so far.

Summary/Conclusions: According to our experience, the implementation of G8 is good prognostic parameter. Its incorporation into standard hematological indices may help in improving the optimal treatment approach decision in elderly patients.

PB1872

A PROSPECTIVE PHASE 2 TRIAL EVALUATING MONOTHERAPY WITH OFATUMUMAB FOR RELAPSED/REFRACTORY SPLENIC B-CELL MARGINAL ZONE LYMPHOMA (MORE TRIAL): SAFETY ANALYSIS RESULTS

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Background: Due to the lack of prospective clinical trials, treatment guidelines for splenic marginal zone lymphoma (SMZL) are mainly based on chemotherapy expertise. Treatment options for progressive disease include splenectomy, chemo-immunotherapy, or anti-viral therapy in HCV-positive cases. As SMZL cells strongly express CD20 molecule, rituximab has been used in patients unfit for chemotherapy or splenectomy with high response rates. Ofatumumab is a fully humanized, high-affinity anti-CD20 monoclonal antibody which can induce a more potent complement-dependent cytotoxicity if compared to rituximab. We designed this multicenter, open-label, single-arm phase 2 trial addressing activity and safety of ofatumumab monotherapy in patients with relapsed/refractory (R/R) SMZL.

Aims: The primary objective is the activity of ofatumumab in terms of complete response (CR) rate. Secondary objectives aim at evaluating the safety and tolerability and exploratory endpoints investigate biological features potentially related with response to ofatumumab.

Methods: All patients provided written informed consent. Key eligibility criteria include R/R disease after ≤2 prior lines of chemotherapy or immunochemotherapy (including single-agent rituximab). Patients are treated with ofatumumab (1st dose: 300 mg, 2nd-8th doses: 1000 mg) up to weekly doses. Response assessment is scheduled 3 months after the last dose. Sample size was defined assuming a P0 of 45% CR, and a P1 of 65% CR. Per Simon optimal Two-Stage design (type I error=0.05, power=80%), 43 patients should be recruited. A safety analysis was planned after the enrollment of the first 10 patients. With an expected rate of adverse events (AEs) of 13%, if less than 3 AEs leading to withdrawal from treatment are reported, the accrual will
continue to the planned 15 patients (interim analysis). Here we present safety analysis results.

Results: Ten patients (6 males, 4 females; median age: 69.5 years, 9 ≥65 years, 1 <65 years) were analyzed for safety. Eight patients were previously treated with rituximab, 26 adverse events (AEs) occurred in 7 patients, with only 5 grade 3-4 AEs. Ten AEs were drug-related, 30% were of grade 3 (Table 1). Three SAEs occurred: hypersensitivity, n=2, both related, dyspnea, n=1, unrelated to study drug. No AEs leading to treatment withdrawal were reported and no patients died on study. Hematological and biochemical abnormalities included: neutropenia (any grade 6 cases, grade 3-4: 4), thrombocytopenia (grade 1-2: 3 cases), lymphopenia (grade 1-2: 2 cases), leukopenia (grade 1-2: 5 cases), 1 case of G3 lymphocytosis (grade 1, at baseline grade 2). 9 cases of ALP increase (all grade 1), 1 case each of AST, ALT and bilirubin increase (all grade 1). Preliminary response assessment in these 10 patients documented 5 CR, 4 Partial Responses (PR), and one patient with progressive disease (PD) at the end of treatment.

<table>
<thead>
<tr>
<th>Drug-related AEs</th>
<th>N of events (any grade)/grade 3-4</th>
<th>Non-drug related AEs</th>
<th>N of events (any grade)/grade 3-4</th>
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<tr>
<td>Treatment-related AEs</td>
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<td>Thrombosis</td>
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<td>Immune-related</td>
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<td>Non-treatment-related AEs</td>
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Summary/Conclusions: Ofatumumab is safe and generally well-tolerated even in elderly patients with R/R SMZL. No cases of unexpected adverse drug reactions were documented. In a series of patients largely pre-treated with rituximab, ofatumumab resulted in a 90% overall response rate, 50% being CR. Complete results of the interim analysis will be presented at meeting.

PB1873

TREATMENT PATTERNS AND RESPONSE TREATMENT IN PATIENTS WITH FOLLICULAR LYMPHOMA IN ROUTINE CLINICAL CARE – A UNITED STATES ELECTRONIC MEDICAL RECORD DATABASE STUDY

A. Galaznik1,*, J. Bell1, L. Hamilton2, A. Ogbonnaya2, K. Hennenfent2, M. Suarez2, A. Mestre2, M. Ferraro1, E. Abella1, L. Martinez1, C. Pedro1, E. Torres1, J. Maiques3, L. Colomo4, C. Besses1, A. Salar1
1Hematology department, Hospital del mar, Barcelona. Grup de recerca aplicada en Hematologia PSMAR, 2Nuclear Radiology department, 3Radiology department, 4Pathology department, Hospital del Mar, Barcelona, Spain

Background: Follicular lymphoma (FL) is an indolent lymphoid B neoplasm corresponding to 20-25% of non-Hodgkin lymphomas (NHL). Bone marrow biopsy (BMB) is part of standard work-up in indolent NHL since up to 40-70% of cases have bone marrow involvement. This fact imposes one factor considered in the FLIPI-1 and FLIPI-2 prognostic index. Positron emission tomography/computed tomography (PET-CT) is a noninvasive technique that shows high sensitivity of detecting nodal and extranodal lymphoma involvement, specially in aggressive subtypes. Some studies have described a high sensitivity (62-100%) and specificity (86-100%) in the detection of bone marrow involvement in aggressive NHL. However, its role in low-grade indolent lymphomas such as follicular lymphoma remains controversial.

Aims: To analyze retrospectively the diagnostic accuracy of PET-CT in comparison with BMB in the initial staging of new FL in a single centre in daily practice.

Methods: One hundred and thirty-six patients with de novo FL have been diagnosed in our institution from June 2005 to October 2016. Of them, 64 who underwent both BMB and PET-CT before treatment were evaluated. The BMB was evaluated by hematologist-pathologist and the interpretation of PET-CT images was interpreted by a nuclear radiologist. Positive BMB was defined as the presence of CD20 +CD10+ y Bcl-2+ lymphoid infiltration. No molecular biology techniques were done in the bone marrow tissue. PET-CT bone marrow involvement was defined as an elevated FDG uptake in the bone marrow than those in liver or mediastinum.

Results: Three-five male and 29 female were included. The median age at diagnosis: 58 years (range 23-84). Thirty-four patients had grade 1-2 FL and 30 grade 3a FL. Bone marrow involvement was diagnosed in 33 of 64 patients (51.1%) by BMB. Out of the 17 patients with positive PET-CT, 4 had negative BMB. Out of 33 patients with positive BMB, 13 had a positive PET-CT (Table 1). The positive and specificity of PET-CT was 39% and 87%, respectively. The positive predictive value and negative predictive value was 76.5% and 57%, respectively.

Table 1. Detection of BMO involvement: BMB and PET-CT results.

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Summary/Conclusions: Our study shows a very low sensitivity of PET-CT in the daily practice. These results contrast with those reported in some recent studies in aggressive lymphoma. However, the high positive predictive value raises the question about the usefulness of BMB in these PET-CT positive cases. In our opinion, with the current data, BMB should be performed in indolent NHL patients.

PB1875

SURVIVAL OUTCOMES AFTER FIRST-LINE THERAPY IN FOLLICULAR LYMPHOMA USING A UNITED STATES ELECTRONIC MEDICAL RECORD-BASED COHORT

A. Galaznik1,*, J. Bell1, L. Hamilton2, A. Ogbonnaya2, A. Raju2, K. Hennenfent2, M. Eaddy2, Y. Shou1
1Millennium Pharmaceuticals, Inc., a wholly owned subsidiary of Takeda Pharm

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PB1875

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1Millennium Pharmaceuticals, Inc., a wholly owned subsidiary of Takeda Pharm
Background: FL is a heterogeneous disease, and clinical presentation is highly variable. The Follicular Lymphoma International Prognostic Index (FLIPI-2) identifies prognostic factors at diagnosis but does not predict in whom and when to initiate first-line therapy (LT).1 Recommended therapies for LT vary by stage, symptomatology, and tumor burden but include monotherapy with rituximab (R) or in combination with other chemotherapies. Survival of FL patients in the R era has greatly improved, but few studies have evaluated survival outcomes in patients seen in routine clinical care.

Aims: This study aimed to evaluate survival outcomes in a US population of newly diagnosed FL patients seen in routine clinical care.

Methods: A retrospective study was conducted in which the presence of ≥1 inpatient record or ≥2 outpatient records with FL diagnosis codes were identified. Newly diagnosed FL patients from Humedica, a large US EMR database, were included from 01/01/08 to 07/31/15. The study index date was the first FL record. Patients who subsequently initiated 1LT for FL were followed from the date of treatment initiation until death, loss to follow-up, or end of study (09/30/15) for the evaluation of survival outcomes. Median progression-free survival (PFS) (defined as initiation of second-line therapy, evidence of supportive care >30 days after the end of a line of therapy, or death), median overall survival (OS), and PFS and OS rates at 2 years following initiation of 1LT were evaluated using Kaplan-Meier analyses.

Results: 1.346 newly diagnosed FL patients who initiated 1LT met the patient selection criteria. 47.7% were male, and the mean age was 65.4 years (SD: 12.7). At baseline, 16.6% of patients had a Charlson Comorbidity Index of ≥2, and the most common comorbidities were diabetes (14.5%) and chronic pulmonary disease (11.2%). 1LT consisted of both monotherapy (38.6%) and combination therapy (61.4%). For monotherapy, R was the predominant agent used (82.3%). For consolidation therapy, bendamustine (88.3%) and R-CHOP (43.8%) were the most common. Kaplan-Meier analysis revealed that the 2-year OS and PFS rates from initiation of 1LT were 86.9% and 64.6%, respectively. Median OS was not reached, and median PFS was 48.1 months (95% confidence interval: 39.4, 58.4).

Summary/Conclusions: The 2-year OS and PFS rates in this newly diagnosed FL patient cohort who received 1LT (the majority of which was R-based) were consistent with expectations in a post-R era. Future analysis will explore the differences in clinical characteristics and survival outcomes for patients who received R monotherapy and various R-combination therapies.

PB1876
Abstract withdrawn.

PB1877
RITUXIMAB MAINTENANCE AFTER R.BENDAMUSTINE FOR PATIENTS WITH UNTREATED FOLLICULAR LYMPHOMA: A REAL LIFE STUDY IN SOUTHERN ITALY ON BEHALF OF RETE EMATOLOGICA PUGLIESI

Background: The Follicular Lymphoma International Prognostic Index (FLIPI-2) identifies prognostic factors at diagnosis but does not predict in whom and when to initiate first-line therapy (LT).1 Recommended therapies for LT vary by stage, symptomatology, and tumor burden but include monotherapy with rituximab (R) or in combination with other chemotherapies. Survival of FL patients in the R era has greatly improved, but few studies have evaluated survival outcomes in patients seen in routine clinical care.

Aims: This study aimed to evaluate survival outcomes in a US population of newly diagnosed FL patients seen in routine clinical care.

Methods: A retrospective study was conducted in which the presence of ≥1 inpatient record or ≥2 outpatient records with FL diagnosis codes were identified. Newly diagnosed FL patients from Humedica, a large US EMR database, were included from 01/01/08 to 07/31/15. The study index date was the first FL record. Patients who subsequently initiated 1LT for FL were followed from the date of treatment initiation until death, loss to follow-up, or end of study (09/30/15) for the evaluation of survival outcomes. Median progression-free survival (PFS) (defined as initiation of second-line therapy, evidence of supportive care >30 days after the end of a line of therapy, or death), median overall survival (OS), and PFS and OS rates at 2 years following initiation of 1LT were evaluated using Kaplan-Meier analyses.

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Summary/Conclusions: The 2-year OS and PFS rates in this newly diagnosed FL patient cohort who received 1LT (the majority of which was R-based) were consistent with expectations in a post-R era. Future analysis will explore the differences in clinical characteristics and survival outcomes for patients who received R monotherapy and various R-combination therapies.

Reference

PB1878
Abstract withdrawn.

PB1879
ROLE OF F-18 FDG-PET/CT IN DETECTING LYMPHOMATOUS BONE MARROW INVOLVEMENT IN THE INITIAL STAGING OF PATIENTS WITH FOLLICULAR LYMPHOMA

Background: The role of F-18 FDG-PET/CT in the staging of newly diagnosed patients with lymphoma was reviewed in the Recommendations of Lugano Classification. They conclude that if a PET/CT is performed, a bone marrow biopsy is no longer indicated for a routine staging of Hodgkin lymphoma (HL) and most diffuse large cell lymphoma (DLBCL). Data are insufficient in follicular lymphoma (FL) and bone marrow biopsy is always recommended.

Aims: We study the value of F-18 FDG-PET/CT for the detection of bone marrow involvement in the initial staging of patients with lymphoma.

Methods: Newly diagnosed patients with HL, DLBCL and FL who underwent F-18 FDG PET/CT and bone marrow biopsy for initial staging between January 2007 and June 2016 were included. We analyze specificity, sensitivity and concordance of PET/CT compared with bone marrow biopsy. In discordant cases, we review if there was any difference in the staging.

Results: 161 patients were included, 69 DLBCL (38 male, 31 female, median age 59 years), 44 HL (24 male, 20 female, mean age 32y), 48 FL (23 male, 25 female, mean age 55y). Four of the 44 patients with HL had bone marrow infiltration in bone marrow biopsy (BMB+) and PET/CT detected bone marrow involvement in all of them. Four patients. PET/CT was positive in bone marrow (BMB+) in 7 of the 40 patients without bone marrow infiltration in bone marrow biopsy (BMB-), these patients had bone marrow lesions on locations other than iliac crest. Six of the 7 patients were in advanced stage regardless of bone marrow involvement and a patient had sternal involvement by contiguity. Seven of the 69 patients with DLBCL had BMB+; 6 patients with DLBCL and 1 patient. DLBCL and FL. PET/CT had detected bone marrow involvement in all of them. Sixty-two patients of 69 DLCL did not have bone marrow infiltration by biopsy(BMB-), but nine of them had BM PET+. Seven of the 9 patients were in stage IV because of extranodal involvement of other organs. One patient had primary bone involvement of jaw and cervical lymph node, and a patient had cervical involvement by contiguity. Fourteen patients of 48 patients with FL had BMB+. Of these 14 patients with bone marrow involvement by biopsy, 5 patients had BM PET+ and PET/CT could not detect another extranodal involvement in three of these five patients. Of the 34 patients without bone marrow infiltration by biopsy BMO- 8 patients had PET-TAC+, and 6/8 could be classified in stage IV regardless of bone marrow involvement (Table 1).

Summary/Conclusions: Our series confirms that PET/CT is useful to detect bone marrow involvement in the initial staging of Hodgkin Lymphoma and DLBCL, we avoid a bone marrow biopsy in these histological variants of lymphoma. In follicular lymphoma, PET/CT did not detect more than one third of patients with bone marrow infiltration by biopsy. These results support the histological assessment of bone marrow in the initial staging of follicular lymphoma.
PB1880

PREDICTIVE FACTORS FOR INFECTIONOUS ADVERSE EVENTS IN PATIENTS WITH B-CELL NON-HODGKIN LYMPHOMA TREATED WITH BENEDANSMUSTINE-RITUXIMAB (R)R MAIN REATNMENT. RESULTS OF A REAL-WORLD EXPERIENCE

A. Di Rocco1, F. De Angelis1, L. Petrucci1, F. Vozzella1, D. De Benedittis1, R. Foà1, M. Martelli1
1Department of Cellular Biotechnologies and Hematology, University of Rome Sapienza, Rome, Italy

Background: The combination of bendamustine (B) and rituximab (R) is an effective and well tolerated treatment for B-cell malignancies. However, previous reports have shown a higher incidence of lymphopenia and secondary infectious complications in patients treated with BR than in patients treated with other chemomunotherapy regimens.

Aims: We performed a retrospective analysis at our institution in patients treated with BR or with or without R maintenance, with the aim of determining the incidence of the infectious adverse events (AEs) and of identifying potential predictors factors.

Methods: We collected data from 65 patients with B-cell non-Hodgkin lymphoma (NHL) who received at least two cycles of BR±R maintenance between 2010 and 2016 at our institution. The AEs – including neutropenia (N), neutropenic fever (NF), lymphopenia, infections episodes and the occurrence of second tumors - were recorded according to the CTCAE v4.0 grade score. We compared the patients who were treated with BR and those who were treated with BR±R. We did not. Univariate analysis with Fisher’s exact test was used to evaluate the potential risk factors.

Results: The median age at the first treatment cycle was 66 years (range 36-89); 33 patients (50%) were ≥65 years, 27 (41%) were male, 53 (82%) had advanced disease, 37 (57%) had bone marrow involvement. Thirty (46%) patients had follicular lymphoma, 17 (26%) mantle cell lymphoma, 11 (17%) marginal lymphoma, 5 (7%) diffuse large B-cell lymphoma and 4% other indolent lymphomas. Thirty two patients (49%) received BR as first line treatment, 51% as second line and above. Bendamustine was administered either at the dosage of 90 mg/m² or 105 mg/m² on days 1, 2 and R was administered at a dose of 375 mg/m² iv or sc, on day 1. Therapy was administered every 4 weeks up to 6 courses. Twenty nine patients (46%) received R maintenance every 8-12 weeks for two years. The mean number of cycles administered was 5 (range 2-10). 33 patients (50%) discontinued treatment due to toxicity: 8/13 for non-hematologic toxicity. Primary or secondary G-CSF prophylaxis was administered to 25 patients (38%), while the prophylaxis with trimetropin-sulfamethoxazole against Pneumocystis jiroveci pneumonia was given to all patients. Twenty two patients (34%) had at least one infection. Bacterial pneumonia was identified in 6/22 patients, varicella zoster virus infection in 4/22, cytomegalovirus reactivation in 2/22 and other infections in 10 patients. At univariate analysis, the infectious AEs were associated only with lymphopenia during the second cycle (p=0.043) and with neutropenia during the second, third and fourth cycle (p=0.026, p=0.003, p=0.018, respectively). No correlation with age, line of treatment and G-CSF administration was documented. Other AEs were: grade 3/4 neutropenic fever (3%), grade 3/4 lymphopenia (80%). We reported also a 5% incidence of second tumors after treatment (lung cancer in 2 patients and prostate cancer in 1).

Summary/Conclusions: In our analysis, BR±R maintenance confirms a toxicity profile similar to that reported in previous experiences. According to our results, an early lymphopenia and neutropenia (after two cycles) are predictive factors for infections AEs and for premature treatment discontinuation. Twenty% of patients discontinued treatment mostly because of the early withdrawal due to infectious complications. These data raise the question on the role of antibiotic, antiviral and primary G-CSF prophylaxis in all patients treated with BR.

PB1881

CAUSES OF DEATH OF FOLLICULAR LYMPHOMAS. MONOCENTRIC AND RETROSPECTIVE STUDY WITH A LONG PERIOD OF OBSERVATION

L. Rigacci1, S. Kovalchuk1, F. Lancia2, G. Manneschi3, B. Puccini1, G. Benelli1, L. Mannelli1, A. Bosi1
1Haematology, AO-Asl Careggi University of Florence, Florence, 2Oncology, Oncology Department, Ferrara, 3Istituto per la Prevenzione Oncologica, ISP, Firenze, Firenze, Italy

Background: Follicular lymphomas are usually defined as incurable diseases with a natural history characterized by continuous relapses.

Aims: This study was launched to evaluate after a long observation period the causes of death during follow-up.

Methods: All patients with histologically confirmed diagnosis of follicular lymphomas grade I-II or IIIa were selected from our data base starting from January 2000 until December 2004 in such a way to have more than 10 years of observation for the patients. We considered all patients with this diagnosis regardless to treatment and considering also patients followed with watch and wait. Patients were followed with ambulatory evaluation and for those lost to follow-up consulting the regional cancer registry.

Results: One hundred and forty-six patients were diagnosed and treated at our institution. The median age at diagnosis was 61 years (range 35-77); stage I-II in 47 patients, III-IV in 86. Bone marrow biopsy was positive in 87 patients, FLIPI 0-1 in 40, FLIPI 2 in 48, FLIPI 3 in 40 and FLIPI 4 in 18 patients. According to treatment 98 patients were treated with antracycline containing regimens, 34 with fludarabine containing regimens and 14 were observed or treated with rituximab 95 patients. 98 patients were treated with 74 or chemotherapy combined in 24; 48 patients did not use rituximab. The median observation period for alive patients was 13.4 years (range 11-15 years) and 8 years (range 0.09-15 years) for dead patients. Sixty-five patients died during this long period of observation and the causes were: 35 due to lymphoma progression (35%), 16 second tumors (16.5%), 12 other disease (18%), 1 car accident and 1 unknown. The overall survival with a median period of observation of 127 months (range 2-196) was 71%. In univariate analysis the best overall survival was statistically associated with low FLIPI score, the use of Rituximab and the obtainment of complete remission. In multivariate analysis both the progression of lymphoma and the obtainment of complete remission maintained the significance. Exactly the same results were observed if we considered the cause specific mortality.

Summary/Conclusions: In conclusion this retrospective monocentric study confirms that after a long follow-up period about half patients died of lymphoma and the other half died for complications related to therapy or to lack of immunological control (second neoplasm or other diseases). Follicular lymphoma confirms to be a good prognosis lymphoproliferative disorders and in the long observation period of patients clinicians must have maintained a careful evaluation of concomitant pathologies.

PB1882

CLINICAL CHARACTERISTICS AND PROGNOSIS OF PATIENTS WITH INDOLENT NON-HODGKIN LYMPHOMA AND RISK OF TRANSFORMATION TO AGGRESSIVE LYMPHOMA: A SINGLE JORDANIAN CENTER EXPERIENCE

D. Zahrani1, M. Ayesh (Haj Yousef)1, T. Kewan1, S. Al Bashir2
1Internal Medicine, 2Pathology, King Abdullah University Hospital (KAUH), Irbid, Jordan

Background: Indolent Non Hodgkin Lymphomas (INHL) are slow growing lymphomas gradually arises from B-cells. They are characterized by slow appearance and progression of symptoms compared to aggressive non Hodgkin lymphoma (NHL) namely Diffuse large B-cell Lymphoma (DLBL). Small percentage of INHL might transform to aggressive NHL.

Aims: We aim to describe the clinical characteristics, prognosis and risk of transformation to aggressive lymphoma in patients with INHL in North Jordan as a model for other Middle East countries in which such data is lacking.

Methods: All patients diagnosed with INHL between Jan 2003 to Jan 2017 were retrospectively reviewed. Clinical and laboratory data at time of diagnosis including gender, age, lactate dehydrogenase level (LDH), pathological subtypes, CT and PET/CT scans were studied. Extranodal involvement was confirmed either by histopathological studies or CT and PET/CT scan. Transformation to aggressive lymphoma was confirmed by histopathological studies. Patients were followed and overall survival rate was calculated. Mean survival times were calculated using Kaplan-Meier method.

Results: Among 265 patients diagnosed with NHL, only 88 patients (33.20%) confirmed to have INHL. 54 patients (61.4%) were males and 34 patients (38.6%) were females. Their ages at diagnosis ranged from (29-83) years with a mean (SD) of 66.7 (12.39). Among these patients, 45 patients (51.1%) had small lymphocytic lymphoma / chronic lymphocytic leukemia (CLL), 20 patients (22.7%) had follicular lymphoma (FL), 15 patients (17%) had marginal zone lymphoma (MZL), 6 patients (6.8%) had mantel cell lymphoma (MCL) and 2 patients (.78%) had unspecified NHL. Mean age of MZL (53.2 years) and FL (55.3 years) were significantly lower than mean age of MCL (58 years) and CLL (62.77 years). 22 patients (23.9%) had extra nodal involvement. There were significant associated differences by slow appearance and progression of symptoms compared to aggressive non Hodgkin lymphoma.

Summary/Conclusions: Prevalence of INHL among patients with NHL in North Jordan is 33.2%. The most common INHL subtypes in our patients were
Summary/Conclusions: The majority of patients in our cohort had favorable outcomes. Currently there is no national guideline for the management of OAL in the UK. Several treatment options exist including chemotherapy, radiotherapy, immunotherapy, observation or more recently the use of eradication treatment for Chlamydia Psittaci. Factors to consider when choosing a treatment include a patient’s co-morbidities, risk of visual impairment, need for systemic therapy, histological diagnosis and anticipated side effects. As treatments are so effective the long term consequences and possible late effects need to be acknowledged and avoided if at all possible. Observation is an acceptable approach in asymptomatic patients when there is no immediate risk of visual impairment. Radiotherapy is an effective first line treatment in symptomatic or localized OAL. The exact role of chemotherapy to achieve disease control with minimal long term side effects is yet to be determined. Reviews with larger number of patients are needed to inform a practical approach to the management of OAL.

PB1884

AGE AS A POTENTIAL NOVEL PROGNOSTIC INDICATOR IN PRIMARY CUTANEOUS B-CELL LYMPHOMA

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Background: Primary Cutaneous B-Cell Lymphoma (PCBCL) comprises a rare group of cutaneous Non-Hodgkin’s lymphomas (NHLs) with an estimated annual incidence of 2.5 per 1,000,000 persons. They usually present with papules or nodules on the head, trunk, and/or extremities. The International Society for Cutaneous Lymphoma (ISCL) and the European Organization for Research and Treatment of Cancer (EORTC) developed a new way to classify PCBCL into three different subtypes. Indolent subtypes include Primary Cutaneous Marginal Zone Lymphoma (PCMZL) and Primary Cutaneous Follicular Center Lymphoma (PFCFL). Primary Cutaneous Diffuse Large B-Cell Lymphoma (PCDLBCL) is an aggressive subtype with a fatality rate of 50%. The Cutaneous Lymphoma International Prognostic Index (CLIP) can risk stratify indolent subtypes, but criteria do not include age. Here we present our single institutional analysis of clinicopathological features and outcomes of patients with PCBCL.

Aims: To analyze clinical and laboratory characteristics such as age, lesion characteristics, hematological parameters, and treatment modalities in order to determine their impact on progression free survival (PFS) in PCBCL.

Methods: A Retrospective cohort of patients with low grade OAL treated in a single Centre between 2003 and 2016 was analyzed. Chemotherapy was the first choice of therapy until 2008, afterwards radiotherapy became the first line treatment for OAL.

Results: A total of 20 patients with OAL were identified. 60% (12/20) of patients were females with a median age of 61.5 years (range 45-85 years). 80% (16/20) had unilateral disease at presentation. MALT lymphomas comprised 75% (15/20), Follicular NHL 15% and CLL/SLL 10%. Only 10% (2/20) had a prior diagnosis of NHL. At presentation 20% (4/20) had evidence of systemic involvement: 19% (3/16) had bone marrow involvement and 1 patient had small volume lymphadenopathy on CT scan. 45% (9/20) were managed under observation. In the chemotherapy group 55% (5/9) experienced 1 relapse (3/5 local recurrence and 2/5 extra-ocular relapse), 3 patients experienced ≥2 relapses, 2 patients had disease transformation to high grade and 1 patient subsequently died as a consequence of their disease. 33% (2/6) patients treated with radiotherapy experienced disease recurrence, mainly extra-ocular and 50% (3/6) suffered complications following radiotherapy in the form of dry eyes and cataract. Median follow up was 9.5 years (range 1-14 years). Overall survival was 95% (19/20) with an event free survival of 65% (13/20) (Table 1).
immunoglobulin heavy chain rearrangement (CDR2 / CDR3 of IgH) in PB, bone marrow and affected organs. All diagnoses were classified according to WHO (2016 revision). In addition, we performed an autonomy test in most patients.

Results: Among the 56 patients, 26 were men (46.4%) and 30 women. The median age at diagnosis was 64 years (37-92). The most frequent subtype was chronic lymphocytic leukemia. The median follow-up was 59 (3-96) months. At the time of diagnosis, 17 patients had splenic marginal zone lymphoma (3 patients, 5.4%). Five of them presented with multifocal disease (8.9%). Fifty percent (28) had a clinical stage III / IV and 32 patients (57.1%) had a low risk of diagnosis (FLIPI 0-1). We found an antigenic stimulus in 11 patients (Helicobacter pylori, Sjögren’s syndrome, Hashimoto’s thyroiditis). The molecular study of MALT1 was performed in 25 patients and 3 presented the translocation (12%). Sixteen of seven cases (35.3%) showed light rearrangements. Antinuclear antibodies were positive in 15 of 32 patients (46.9%). In 46 patients (82.1%) a complete remission (CR) (76.1%) and 10 partial remission (PR) in 3 (30%) after rescue treatment. There was just one case of high grade transformation (1.8%), who was the only patient deceased in the series (1.8%), with a median follow-up of 70 months.

Summary/Conclusions: Marginal zone lymphoma is an indolent disease with a good prognosis and very good response to current therapy. It is sometimes associated with autoimmune phenomena and infectious agents. It is essential a correct staging and characterization to optimize its therapeutic management and outcome.

PB1886

HAIRY CELL LEUKEMIA AND B-RAF MUTATIONS

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Aims: To investigate the frequency of B-RAFV600E mutation and other rare mutations of B-RAF (B-RAFG464E, B-RAFG466E, B-RAFG469E) and their relation with clinical data and treatment responses.

Methods: Charts of 13 patients diagnosed with HCL were analyzed retrospectively. Patients’ clinical parameters were evaluated. HCL variant type patients were excluded. Paraffin blocks of spleen or bone marrow tissues are obtained from the pathology archives. One thin section (10 micron) of bone marrow or three sections of spleen are cut and DNA extracted by spin column technique using DNA extraction kit. (QiAamp DNA FFPE Tissue Kit, Qiagen) After spectrophotometric measurement of DNA; common and uncommon mutations of B-RAF were investigated. (Qiagen PyroMark Q24 system, Therascreen Braf Pyrokit 24, V1 (1/2) kit) Mutation and clinical data analysis were conducted using the SPSS 15.0 software. The study was approved by the local ethics board of Dokuz Eylul University.

Results: 41 patients were valid for analysis. Characteristics: 70% males with a mean age of 62 years (30-87). ECOGs 2 in 95% of cases, 73.2% in stages III-IV and FLIPI ≥3 in 48%. Bulky mass in 13% of patients, LDH and β2-microglobulin increased by 12% and 41.2% respectively and bone marrow infiltration was seen in 60%. 88% of patients had received one line of treatment. One B-RAF V600E mutation was demonstrated in 11/100 of Tacci HCL case series. B-RAFV600E mutation was positive in 10 (%76.9) patients. Three patients (12%) showed other mutations (two B-RAF464E, one B-RAF466E, one B-RAF469E). Two patients were positive for both mutations. No relation could be determined between clinical findings and mutation state.

Summary/Conclusions: B-Raf mutations are variable and common mutations in HCL patients. B-RAFV600E mutation testing can be used as a supportive test for the diagnosis of HCL due to high incidence of mutation. Also it can be used as an indicator for patient selection that are appropriate for target therapies.

PB1887

BENDAMUSTINE-RITUXIMAB IN PATIENTS WITH RELAPSED FOLLICULAR LYMPHOMA PREVIOUSLY EXPOSED TO RITUXIMAB. EXPERIENCE IN SEVEN HOSPITALS OF THE SPANISH GELTAMO GROUP

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Aims: To evaluate the efficacy and safety of the bendamustine-rituximab association in a group of patients with follicular lymphoma previously exposed to rituximab.

Methods: Retrospective analysis of patients with relapsed FL treated with BR in 7 spanish hospitals on behalf of the Spanish Lymphoma Group (GELTAMO). The study was approved by the reference Ethnic Committee and by all of the participating centres. All patients acceded to the treatment through the compassionate use program.

Results: 41 patients were valid for analysis. Characteristics: 70% males with a mean age of 62 years (30-87). ECOGs 2 in 95% of cases, 73.2% in stages III-IV and FLIPI ≥3 in 48%. Bulky mass in 13% of patients, LDH and β2-microglobulin increased by 12% and 41.2% respectively and bone marrow infiltration was seen in 60%. 88% of patients had received one line of treatment. One B-RAF V600E mutation was demonstrated in 11/100 of Tacci HCL case series. B-RAFV600E mutation was positive in 10 (%76.9) patients. Three patients (12%) showed other mutations (two B-RAF464E, one B-RAF466E, one B-RAF469E). Two patients were positive for both mutations. No relation could be determined between clinical findings and mutation state.

Summary/Conclusions: BR has a high efficacy and a good safety profile in this series of patients with relapsed FL previously exposed to rituximab. The number of previous treatments (1 vs ≥2) and the age had no impact in the results.

PB1888

USE OF RADIATION THERAPY FOR THE TREATMENT OF GASTRIC MALT LYMPHOMA

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Background: Gastric mucosa-associated lymphoid tissue (MALT) lymphoma is a rare disease however, the incidence is increasing and closely associated with helicobacter pylori (HP) infection. One choice of treatment of gastric MALT lymphoma refractory to HP sterilization is radiotherapy.

Aims: Our aim was to analyze the response to treatment with definitive radiotherapy in our department.

Results: Between January 2014 and January 2017, 8 patients with gastric MALT lymphoma were treated with eradication therapy of HP, followed by definitive radiotherapy. The average total dose was of 38 Gy to the stomach in a once-daily schedule. Follow-up included computed tomography scan and
endoscopy with biopsies at regular intervals. The median follow-up was 14 months.

Results: In all patients we got complete responses (CR) with no tumor detectable by endoscopy or biopsy after initial treatment, but after 2 years one of them relapsed and required immunochemotherapy. The most common acute toxicities were fatigue and nausea, in our patients. In any case late toxicities were observed. The overall survival was 100% after 2 years.

Summary/Conclusions: In selected patients who are not responsive to HP sterilization, definitive radiotherapy can be an efficient therapy with tolerable complications, preservation of stomach and sustained response over time.

In all patients we got complete responses (CR) with no tumor detectable by endoscopy or biopsy after initial treatment, but after 2 years one had Mixed Phenotypic Leukemia while 1 had Myeloid Sarcoma. The mean interval between diagnosis of tuberculosis and acute leukemia was 37.2 weeks, 10 patients had Acute Myeloid Leukemia, 7 had Acute Promyelocytic Leukemia, 5 had Acute Lymphoblastic Leukemia, 2 had had Acute Myeloid Leukemia while 1 had Myeloid Sarcoma. The mean interval between diagnosis of tuberculosis and acute leukemia was 37.2 weeks, with 2 patients being diagnosed after completion of therapy of acute leukemia.

Background: Lipegfilgrastim (Lonqex®) is a long-acting fixed-dose glycopeylated granulocyte colony-stimulating factor administered once per chemotherapy cycle. It has been available in Europe since 2013. It was proven to be non-inferior with regard to duration of severe neutropenia compared with pegfilgrastim in breast cancer patients. However, data in patients with hematological malignancies are limited.

Aims: We aimed to evaluate the effectiveness of lipegfilgrastim in the cycle following the first lipegfilgrastim-supported treatment cycle in lymphoma patients.

Methods: This is a prospective observational cohort study. Patients with different tumor types treated with cytotoxic chemotherapy (CT) who received lipegfilgrastim in primary prophylaxis (PP) or secondary prophylaxis (SP) are being included in this study. CT dose modifications and neutropenia-related events are recorded and analyzed. Evaluation of effectiveness in the cycle following the first lipegfilgrastim-supported CT cycle in a lymphoma subpopulation is presented here.

Results: At the time of the interim analysis (December 2016), 249 patients diagnosed with lymphoma have been included. Mean age±standard deviation of lymphoma patients was 61.6±15.6 years and 56.6% were male. For the majority of patients (81.1%), intended use of lipegfilgrastim was in PP. Exposure to lipegfilgrastim has been documented for 228 patients with an average of 4.76 cycles per patient. Data on CT dose modifications and neutropenic events following the first lipegfilgrastim-supported cycle were available for 144 and 167 patients, respectively. CT dose was never omitted. CT dose delays were observed in 8.0% (PP) and 18.8% (SP) of patients and CT dose reductions in 4.5% (PP) and 12.5% (SP) of patients. In the first lipegfilgrastim-supported cycle, febrile neutropenia was recorded in 4.5% (PP) and 3.0% (SP) of patients; severe neutropenia was recorded in 7.5% (PP) and 9.1% (SP) of patients. Throughout the treatment, 22 (9.6%) patients exposed to lipegfilgrastim reported at least 1 adverse drug reaction (ADR). The most common ADRs were myalgia and musculoskeletal pain. Serious ADRs were reported by 11 (4.8%) patients.

Summary/Conclusions: Lipegfilgrastim is effective and well tolerated in the real-world setting in lymphoma patients, administered either in PP or SP. The results suggest that lipegfilgrastim administered in PP might give better outcomes in terms of dose delays and dose reductions than when administered in SP.

Background: Patients with acute leukemia represent an immune-compromised population, with innate, humoral and cellular immune paresis. These patients are thus at high risk of development of new infections and reactivation of chronic infections. Despite the high prevalence of tuberculosis in the general population in endemic countries, it is rarely suspected and diagnosed in patients with acute leukemia.

Aims: To study the clinical manifestations of tuberculosis in patients with acute leukemia, as well as the impact of infection in the management of leukemia.

Methods: A hospital database search was done to identify cases of acute leukemia and tuberculosis between a study duration of January 2013 to January 2017. All the medical records of the identified cases were retrieved from the central record department. A systemic analysis of characteristics pertaining to acute leukemia, treatment regimen, chemotherapy response, site of tubercular infection, mode of diagnosis and treatment response to anti-tubercular therapy was conducted.

Results: A total of 25 patients with acute leukemia were identified who were also diagnosed with tuberculosis. 10 patients had Acute Myeloid Leukemia, 7 had Acute Promyelocytic Leukemia, 5 had Acute Lymphoblastic Leukemia, 2 had Mixed Phenotypic Leukemia while 1 had Myeloid Sarcoma. The mean interval between diagnosis of tuberculosis and acute leukemia was 37.2 weeks, with 2 patients being diagnosed after completion of therapy of acute leukemia.
and one patient was diagnosed post mortem. The most common organ involved was the lung, which was seen in 80% of patients and 20% of patients had disseminated tuberculosis. The development of tubercular infection led to alteration of therapy for the acute leukemia in 24% of cases, while it was postponed in 44% of cases. In particular, hypomethylating agents were used successfully in two patients with AML as bridge therapy to high dose chemotherapy. 76% of patients were cured of tuberculosis, while 1 patient expired due to tuberculosis and 3 patients could not receive adequate therapy for tuberculosis. 3 patients went on to undergo HSCT post treatment for tuberculosis, and none had a flare of the disease post transplant. 

Summary/Conclusions: The presence of tuberculosis infection in patients of acute leukemia has an impact on the overall management of the patient and strategies such as utilization of hypomethylating agents as bridge therapy may help in successful management of the leukemia. A high index of suspicion is required to suspect and diagnose the presence of tuberculosis as the manifestations are more commonly attributed to fungal infections or to the leukemia per se. These patients usually have a favorable response to empirical antituberculous therapy and the presence of tuberculosis infection does not forego treatment options such as HSCT or high dose chemotherapy for these patients.

PB1891
INCIDENCE OF BACTEREMIA BY MULTI-RESISTANT BACTERIA IN HEMATOLOGY PATIENTS. A DESCRIPTIVE EPIDEMIOLOGIC STUDY FROM A THIRD LEVEL HOSPITAL
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Background: In recent years the incidence of multi-resistant bacteria (MRB) infections have notably increase. These infections are especially serious in hematological patients because of the immunosupression derived from their illness and their treatments. This increase is related to a high mortality rate and high health costs due to the severity of the infections and the difficulty in setting adequate therapy due to the lack of new antibiotics against these pathogens.

Aims: Define the MRB infections incidence and ways of presentation. As a secondary goal we try to determine if the isolation of these MRB has affected our empiric antibiotic therapy decision.

Methods: We retrospectively collected all positive blood stream cultures from hematologic patients from January 2012 to December 2016. We studied the characteristics, clinical features and pathogen isolates of our patients when the blood cultures were obtained.

Results: 1005 positive blood stream cultures were collected in 382 patients. The main characteristics of the patients are shown on Table 1.

Table 1.

The infection source was: central venous catheter (CVC) in 48% of patients (including tunneled and non tunneled and PICC lines), respiratory 10%, abdominal 8%, urinary 5%, skin/soft tissue 7% and multiple location 5%. Regarding CVC isolation’s, 11% were interpreted as contamination and 6% as colonization. Gram positive (G+) bacteria were more frequently isolated than Gram negative (G-) (72% vs 24%). Most common G+ bacteria was coagulase negative Staphylococcus and G- E. Coli, Klebsiella sp and Pseudomonas aeruginosa. MRB were detected in 6.1% of blood cultures being the most frequent G- (85%).

The main resistance mechanism was extended-spectrum beta-lactamases (ESBL) and carbapenemases (CP) production (Table 2). BMR infections increased significantly in last year, mainly associated to CP, 0.5% in 2012 up to 7.1% in 2016 (Figure 1). 29.5% of MRB were multiresistant in in patients and identified as chronic carriers of multiresistant organisms and 100% of them had extensive exposure to wide spectrum antimicrobials previously. 14% of infections began with a serious illness (persistent hyperthermia, hemodynamic disbalance and worsening), 5% needed intensive care assistance and 15% died in any case. In MRB infections 6% were cured, 8% received colistin and carbapenems in extended infusion) was started in 15% of patients, all with serious illness at diagnosis.

PB1892
INFECTIONS PRESENTING IN THE BONE MARROW IN HIV POSITIVE PATIENTS AND THEIR MORPHOLOGICAL ASSOCIATIONS – SIX YEAR DATA FROM AN INDIAN TERTIARY CARE HOSPITAL
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Background: Centre of Disease Control enlists more than 20 infections considered as Acquired Immunodeficiency Syndrome (AIDS) defining. Progression of the disease and falling CD4 counts are the most important risk factors in acquiring these infections. Most of the cases present with non specific symptoms including fever, respiratory and gastrointestinal manifestations. A bone marrow examination is helpful in establishing the diagnosis in many of these cases.

Aims: The aim of this study was to evaluate the incidence of marrow infections in HIV positive patients and to study their morphological spectrum.

Methods: This is a six year retrospective study carried out in a tertiary care hospital in North India. All bone marrow aspirates and biopsies from HIV positive patients were retrieved and evaluated for the presence of infectious etiology. Cytochemical stains like Acid Fast Bacillus, Periodic Acid Schiff, Gomori Methenamine Silver and Mucicarmine were performed wherever needed. The associated morphological features which may assist diagnosis were noted.

Results: Bone marrow samples (either aspirates or biopsies or both) were available in 185 HIV patients. Out of these, fifty three cases (33.5%) were associated with infections. The most common infection in these patients was Mycobacterium Tuberculosis (22.7%). In addition, five cases of Histoplasmosis (2.7%), two cases of Cryptococcosis, two cases of Leishmanina donovani, and one case of Plasmodium falciparum, Parovirus and Microfilaria each were noticed. The morphological spectrum associated with infections in these cases included lymphoplasmacytic infiltrate (68%), granulomas (66%), macrophage infiltration, hemophagocytosis, gelatinous marrow transformation and marrow hypoplasia. Two cases of M tuberculosis were associated with maturation arrest in the bone marrow. One case of Tuberculosis was associated with Non Hodgkin’s Lymphoma. Myelodysplasia was seen in association with Leishmanina infection.
**Summary/Conclusions:** A wide spectrum of infections may be observed in HIV positive patients in the bone marrow. Bone marrow aspirate and biopsy are essential, rapid and cost effective techniques to arrive at the right diagnosis in such cases. Features like hypoplasia, myelodysplasia and maturation arrest may be attributable to infections.

**PB1893**

**UTILITY OF BONE MARROW BIOPSY IN FEVER OF UNKNOWN ORIGIN: A CRITICAL ANALYSIS OF A RETROSPECTIVE SERIE**

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**Background:** The utility of bone marrow biopsy trephine (BMT) as a diagnostic tool in patients with fever of unknown origin (FUO) is a subject of controversy and debate. BMT has been shown to be safe and useful in patients with HIV/AIDS but its value in immunocompetent patients has not been sufficiently assessed. It's reported the use of diagnostic BMT as a rapid decision-making tool in patients with FUO and FBU in the proper clinical setting. A BMT demonstrated infection-related evidence prior to positive bone marrow culture in 75% of cases. Special stains and blood cultures had similar diagnostic yield, but BMT offers faster results. Thus, this procedure assists in clinical decision-making and the refinement of treatment in a more timely manner.

**Aims:** To determine the utility of bone marrow biopsies in FUO patients.

**Methods:** We reviewed retrospectively the bone marrow biopsy results of the patients who underwent BMT from January 2010, to December 2016. Demographic, laboratory, diagnostic and outcome data were collected and retrospectively analyzed. We identified 31 patients who fulfilled the accepted classic Peto’s criteria for FUO. The cohort included immunocompromised and immunocompetent patients.

**Results:** The BMT contributes to the diagnosis in only four cases (12.9%). In two patients (6%) the histology revealed the presence of granuloma and/or lymphohistiocytic aggregates; one secondary hemophagocytosis (3.2%) and one mastocytosis infiltrate (3.2%). Six patients had a previous diagnosis of HIV/AIDS (19%). Sub analysis in HIV/AIDS patients revealed positive BMT culture in 2 of the patients (6.4%). Cultures demonstrated Mycobacterium tuberculosis and Mycobacterium avium intracellulare. There was one case in which a pathogen was grown in culture but that had a negative of ‘direct examination’. The associations most likely related factor to contribute to the diagnosis in HIV/AIDS was male predominance (58% odds ratio [OR] 2.95; 95% CI, 1.19-4.25), clinical lymphadenopathy (OR 4.97; 95% CI, 1.90-2.44) or anemia (OR, 2.21; 95% CI, 1.26-3.84). Reactive myeloid hyperplasia was represented 15 cases (48%). Non- haematological diagnosis (lymphoma, Leukemia) was made on the exclusive bases of biopsy results.

**Summary/Conclusions:** Bone marrow examination is an integral part of investigation of FUO, however, morphological finding alone would not be sufficient to ascertain the diagnosis. In present study only two cases of established infections were revealed. Both were present in HIV/AIDS. These results are explained because a highly active antiretroviral therapy has reduced incidence of opportunistic infections. The percent of opportunistic infections diagnosed by BMT was very low and did not justify an invasive procedure. The presence of granulomas in trephine biopsy incriminated for establishing the diagnosis of lymphoproliferative malignancy. Both bone marrow biopsy is still a useful ancillary procedure for establishing the diagnosis of FUO, however, morphological finding alone would not be sufficient to ascertain the diagnosis.

**PB1894**

**THE OUTCOME OF PEDIATRIC CANCER PATIENTS ADMITTED TO THE INTENSIVE CARE UNIT OF A TERTIARY HOSPITAL IN GWANGJU-CHONNAM, KOREA**

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**Background:** Recent advances in supportive care have considerably improved the prognosis of pediatric cancer patients. However, the use of aggressive cancer treatment is also associated with complications and life-threatening events that result in admissions to the intensive care unit (ICU).

**Aims:** This study aimed to analyze the outcome of pediatric cancer patients admitted to the ICU.

**Methods:** A retrospective analysis of 84 ICU admissions of cancer patients <21 years old between May, 2004 and Aug. 2016 at Chonnam National University Hwasun Hospital (CHNUH) was undertaken. The risk factors for short-term outcome (survival at the time of discharge from the ICU) were analyzed. After excluding scheduled perioperative admissions, the records of 81 admissions (75 patients) were reviewed.

**Results:** Hematologic cancer patients represented 71.6% of admissions. The mean duration of ICU stay was 10.7 days. Respiratory failure (39.5%) and septic shock (17.8%) were the most frequent indications for ICU admissions. Overall mortality rate was 46.9%. The mortality for hematologic cancer was 51.7% as compared to 34.8% for solid cancer (P < 0.05). Mortality for individual indication was as follows: bleeding, 66.7%; respiratory failure, 59.4%; systemic infection 57.5%, anterior mediastinal syndrome, 50%, neurologic disorders, 37.5%, renal disorder, 37.5%, and so on. ICU mortality after hematopoietic stem cell transplantation was 66.7%, mostly within 100 days post-transplant. The median Pediatric Risk of Mortality Score (PRISM) III score of survivors was lower than that of non-survivors (11.3±5.1 vs 19.9±10.8, P < 0.001). The mortality rates were 70.3% and 27.3% in patients with high (>15 points) and low (<15 points) PRISM III score, respectively (P < 0.001). Mortality rate was significantly related to the presence and number of organ system dysfunction (P < 0.01 and P < 0.001, respectively), positive inotropic support (P < 0.01), and mechanical ventilation (P < 0.001). By using multivariate logistic regressions, the independent risk factors were mechanical ventilation (OR, 8.0; 95% CI, 1.7-21.3; P < 0.01), and organ system dysfunction (OR, 18.5; 95% CI, 4.4-77.0; P < 0.001). Hematologic cancer patients had higher mean PRISM score (16.6±9.4 vs 12.2±8.6; P = 0.51) and higher risk of sepsis (39.3% vs 13.0%; P < 0.001) compared to solid tumor patients.

**Summary/Conclusions:** These results revealed the current status of ICU care for pediatric cancer patients in a tertiary hospital in Korea. Further improvement of supportive care and earlier effective intervention should be translated in gradual reduction in mortality rate in these population.

**PB1895**

**EFFICACY AND SAFETY OF TIGECYCLINE IN FEBRILE NEUTROPENIC PATIENTS WITH HEMATOLOGIC MALIGNANCIES AND CARBAPENEM RESISTANCE: A MULTICENTRE RETROSPECTIVE STUDY FROM CHINESE PEOPLE**

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**Background:** Tigecycline has broad spectrum activity against multidrug-resistant (MDR) bacteria, but few investigations of tigecycline in febrile neutropenic (FN) patients with malignancy are available.

**Aims:** This study attempts to investigate the efficacy and safety of tigecycline in FN and carbapenem resistant patients with hematologic malignancies.

**Methods:** The study of 109 patients with hematologic diseases and FN were retrospectively analyzed. They are unresponsive to carbapenems for 3~5 days before receiving tigecycline (loading dose 100 mg; then 50 mg every 12 hours). Clinical response to treatment was defined as clinical cure, improvement or failure. Meanwhile, the adverse events were documented.

**Results:** The medium duration of neutropenia was 15 days (ranged from 1 to 83d). Out of 109 patients, 96 (88.1%) had respiratory infection, while 33 (30.3%) had bloodstream infection. The total response rate of tigecycline was 65.1%. The bacterial eradication rates and bacterial, hypothetical eradication were 25.9% and 24.1%, respectively. The clinical effective rate was 85.7% when tigecycline was administered for more than 9 days, while just 48.3% when administered for less than 9 days (p<0.001). Patients with bloodstream infection got a worse efficacy than those without (41.2% vs 69.6%, p=0.024). For patients whose absolute neutrophil counts were less than 0.1 x 10⁹/L, the clinical effective rate decreased obviously (59.8% vs 86.4%, p=0.019). The side-effects were well tolerated. No lethal adverse events were observed.

**Summary/Conclusions:** Our results demonstrated tigecycline was effective and safe for patients unresponsive to carbapenems with FN, combination and prolonged duration of tigecycline is recommended, and these results need to be further studied.
Methods: A retrospective cytological study of bone marrow aspirates from 95 patients with HMS (n=27), HMS+HIV (n=8), HMS+HCV/HEV (n=11) and HMS+intestinal parasitosis (n=49) has been performed.

Results: Bone marrow cellularity was normal in all the groups studied except in HMS+HIV patients, in which the cellularity was very diminished (statistically significant difference, p<0.01). Most frequent alterations observed in all samples (HMS and HMS+other entities) that could define the HMS-bone marrow cytological pattern, were: - Erythroid hyperplasia with dyserythropoiesis, which is reflected in a decreased myeloid-erythroid ratio. - Increased eosinophils percentage. - Increased lymphocytes percentage. - Increased plasma cells percentage and detection of Mott cells in a significant proportion of samples from all series (48.1% of HMS samples). Quantitative results for these variables are summarized in Table 1. Lymphocytosis was significantly increased in HMS+HIV/HCV bone marrow (p=0.04). Significant detection of atypical lymphocytes (>4%) varied widely between the groups, ranging from 14.8% of HMS bone marrows to 75.0% of HMS+HIV bone marrows (statistically significant difference, p<0.01). There was no lymphoma evidence in any case. No quantitative or qualitative alterations were detected in megakaryocytes, except for a slight decrease in HMS+HIV bone marrows (statistically non-significant difference) (Figure 1).

Table 1. Quantitative results (mean±standard deviation).

<table>
<thead>
<tr>
<th>Reference values</th>
<th>HMS</th>
<th>HMS+HIV</th>
<th>HMS+HCV</th>
<th>HMS+HEV</th>
<th>HMS+IP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloid-erythroid ratio</td>
<td>3.11</td>
<td>2.61</td>
<td>2.51</td>
<td>3.41</td>
<td>2.51</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>7</td>
<td>12.00</td>
<td>9.00</td>
<td>9.00</td>
<td>12.36</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>37</td>
<td>37.69</td>
<td>37.69</td>
<td>37.69</td>
<td>37.69</td>
</tr>
<tr>
<td>Plasma cells (%)</td>
<td>54</td>
<td>68.07</td>
<td>81.07</td>
<td>64.61</td>
<td>73.33</td>
</tr>
</tbody>
</table>

*Reference standard by the Modified Alvarado Scoring System, Total scores were 10, Score 1-4: acute appendicitis very unlikely; Score 5-7: acute appendicitis probable; Score 8-10: acute appendicitis definite. #: - negative; #: + positive.

Summary/Conclusions: Acute appendicitis occurring during the neutropenic phase in HSCT patients could be diagnosed by the MASS and ultrasonography, and such cases could be cured by conservative therapy. This study could provide a further choice for the diagnosis and treatment of acute appendicitis in leukemia patients of HSCT.

PB1897

ACUTE APPENDICITIS IN LEUKEMIA PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION DURING THE NEUTROPENIC PHASE

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Background: Infectious complications arising from the gastrointestinal tract is common in neutropenic patients with hematologic malignancies, especially during ESHT.

Aims: Sequential analysis of 776 HSCTs in single center, totally 10 cases of acute appendicitis were found out, the treatment and outcome were further analyzed.

Methods: The HSCT patients who occurred acute appendicitis during - 10d~+60d in the Hematological Department of Nanfang Hospital from Jan. 2005 to July 2016 were analyzed. Patients were enrolled in our study based on the Modified Alvarado Scoring combined with ultrasonography (the MASS total score of 1-4: acute appendicitis very unlikely; Score 5-7: acute appendicitis probable; Score 8-10: acute appendicitis definite. We selected those cases with scores 5-10). And the follow-up lasted for 24 m.

Results: 776 HSCT patients were analyzed, in which 10 patients (7 male, 3 female) had acute appendicitis during -1d~+7d, which included two cases of sepsis. The median age was 18.5 (10-39) years of age. 7 patients were ALL and the other 3 were CML. All patients underwent allo-HSCT. 6 patients received conditioning regimen of [DA/ITBI/CYVP-16, and 4 others were treated with [BT/BiCYP-16/Ara-c, FA/BiCYP-16, BuCy and BuCy/Ara-c respectively. All the cases scored range from 5 to 10 of the MASS, and 6 patients showed positive findings on ultrasonography. All the patients had a mean value of neutrophil of 0.06×10⁹/L (Table). 10/10 cases were cured with conservative therapy, mainly contained different kinds of full dose broad-spectrum antibiotics, such as ceftazidime/tazobactam, imipenem, ceftriaxone, meropenem, tigecycline, et al. Within 24 m, 8 patients did not relapse, one patient died from gastrointestinal bleeding 2 m after without recurrence of appendicitis, while one patient relapsed 1 year later and was cured by appendectomy.

Table 1. Compared with the Modified Alvarado Scoring and ultrasonography to diagnose appendicitis during the neutropenic phase of HSCT.

<table>
<thead>
<tr>
<th>Symptoms*</th>
<th>Signs*</th>
<th>Total</th>
<th>Excrussion</th>
<th>Blood Routine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>Abdominal pain</td>
<td>10</td>
<td>5</td>
<td>0.00</td>
</tr>
<tr>
<td>Nausea</td>
<td>Vomiting</td>
<td>5</td>
<td>2.5</td>
<td>0.00</td>
</tr>
<tr>
<td>Anorexia</td>
<td>Tenderness</td>
<td>5</td>
<td>2.5</td>
<td>0.00</td>
</tr>
<tr>
<td>Teralax</td>
<td>Localized turgidity</td>
<td>5</td>
<td>2.5</td>
<td>0.00</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>10</td>
<td>5</td>
<td>0.00</td>
</tr>
</tbody>
</table>

*Reference standard by the Modified Alvarado Scoring System, Total scores were 10, Score 1-4: acute appendicitis very unlikely; Score 5-7: acute appendicitis probable; Score 8-10: acute appendicitis definite. #: - negative; #: + positive.

Summary/Conclusions: Acute appendicitis occurring during the neutropenic phase in HSCT patients could be diagnosed by the MASS and ultrasonography, and such cases could be cured by conservative therapy. This study could provide a further choice for the diagnosis and treatment of acute appendicitis in leukemia patients of HSCT.

PB1898

EPIDEMIOLOGY OF BLOODSTREAM INFECTIONS IN NEUTROPENIC AND NON-NEUTROPENIC PATIENTS WITH MALIGNANCY

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Background: Blood stream infections (BSI) in patients with malignancies remain associated with significant morbidity and mortality. The choice of an empirical antibiotic regimen is usually based on the local epidemiology of the microorganisms and their antibiotic susceptibility profile. Antimicrobial guidelines for the management of sepsis in cancer patients in East Sussex Healthcare Trust (ESHT) recommend piperacillin/tazobactam as monotherapy and gentamicin is added in case of septic shock. Vancomycin is also added as a first line therapy if there is a suspicion of central line sepsis. Alternative therapies are ceftazidime or meropenem plus aminoglycoside.

Aims: We intend to review the aetiology of BSI and check the effectiveness of the antibiotics used in ESHT in cancer patients.

Methods: This retrospective study was conducted at ESHT from January 2006 to December 2015. Demographic and laboratory data were collected from the Pathology information system.

Results: A total of 640 episodes of BSI occurred in 297 patients (159 male). Of the 297 patients, 239 had haematology malignancies while 54 had solid organ tumour. Four patients had both. The neutrophil count was <1 cells/10³ in 383 episodes and majority of BSI occurred in this group. A total of 802 organisms (477 and 325 organisms from neutropenic and non-neutropenic respectively) were isolated. Of 802, 406 Gram positive and 386 Gram negative organisms were isolated. Seven Mycobacterium species and three Candida species were isolated. Most common organisms in neutropenic patients were Coagulase negative Staphylococcus (CoNS) (22%), Klebsiella species (14%), Escherichia coli (13%), Streptococcus species (10%), Pseudomonas species (10%), Enterococcus species (8%) and Staphylococcus aureus (4%). In non-neutropenic patients, CoNS (29%), Escherichia coli (11%), Pseudomonas species (8%), Streptococcus species (7%), and Klebsiella species (5%) were isolated. Twelve Glycopeptide resistant Enterococci were isolated. Four Methicillin resistant Staphylococcus aureus were isolated. In addition, 15 Extended Spectrum Beta-lactamase producing Gram negative bacilli were isolated. Among Gram negative organisms, more than 91% isolates were sensitive to piperacillin/tazobactam, cefazidime and ciprofloxacin and higher sensitivity rates (>96%) were recorded in gentamicin and meropenem, Table1 summarises the effectiveness of antibiotics used.

Summary/Conclusions: This study examined an on-going trend towards Gram positive organisms causing BSI in cancer patients. The antimicrobial regimens used in ESHT are highly effective against commonly isolated organisms. An early diagnosis and timely administration of appropriate antibiotics are imperative in managing BSI. The identification and the antimicrobial susceptibility of the microorganisms causing BSI in cancer patients remain important to develop antimicrobial treatment strategies, and to prevent the spread of antimicrobial resistance.
PB1899

CHANGING TREND IN LOCAL BACTERIAL EPIDEMIOLOGY: EXPERIENCE IN ACUTE LEUKEMIA PATIENTS HOSPITALIZED IN SINGLE HEMATOLOGY UNIT

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Background: The intense chemotherapeutic regimens and hypomelittent agents to treat acute leukaemia induce prolonged neutropenia with high risk of infections.

Aims: To analyze local microbial epidemiology we studied patients admitted to our ward.

Methods: All 100 cases of Acute Leukemia (AL) admitted in our ward from August 2013 to February 2017 received prophylactic antibacterial therapy with fluoroquinolones and were analyzed for weekly routine tissue culture screening and serial blood culture for fever. Six patients were LymphoidAL and 94 were Myeloid AL. 41 patients were not eligible for intensive chemotherapy (for age and comorbidities) and were treated with hypomelittent agents, while 59 were younger than 65 years and were treated with induction / consolidation chemotherapy 3 plus 7 regimen. Median age was 58 years with range from 27 to 88 years old.

Results: We found 28 patients (28%) bacterial septic shock during fever, of which 20 cases gram negative (71%) in particular 65% E.Coli, 15% Enterobacter, 10% Klebsiella, 5% Stenotrophomonas, 5% Pseudomonas; while 8 patients (29%) had a gram positive septic shock (S.Haemolyticus 38%, S.capitis 25%, S. hominis 25%, S.epidermidis 12%). During intensive chemotherapy and prolonged severe neutropenia we took over the major incidence of septic shock (23 patients 82%) than hypomelittent treatment in particular decitabine (5 patients 18%). During 2014 we had 3 mortal septic shock for multiresistant gram-klebsiella and Pseudomonas. Since than we adopted in our ward, isolation of patients with gram negative (klebsiella or pseudomonas ) to issue culture positive, hygienic and sanitary practices with closing room for 48 hours and hand disinfection before entering and after leaving any patients room. We noticed a change of bacterial infections incidence in these 3 years in our ward...reduction klebsiella/pseudomonas multiresistant infections and emergency of E.coli and Staphilococcus septic shock not multiresistant.

Summary/Conclusions: More epidemiological analysis in several haematological ward are necessary to understand if it is a changing local microbial epidemiology or is the different management of neutropenic patients with acute leukemia and/or a different antimicrobial strategy to determine this changing trend.

PB1900

UK SINGLE-CENTRE SERVICE EVALUATION TO DESCRIBE THE IMPACT ON HEALTHCARE RESOURCE USE OF LOCAL ANTIFungal PROPHYLAXIS AND TREATMENT PROTOCOLS IN THE MANAGEMENT OF HIGH-RISK PATIENTS WITH NEUTROPENIA

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Background: Patients with neutropenia, including those with haematologic malignancies, are at high risk of invasive fungal infections (IFI). Pre-2014, there were no formal written guidelines but the guidance at Poole Hospital NHS Foundation Trust specified the use of posaconazole oral suspension for primary prophylaxis in all high-risk patients except those with acute lymphoblastic leukaemia (ALL). In 2014 formal guideline changes included the introduction of the tablet formulation of posaconazole, use of micafungin as first line empirical therapy and a focus towards improving diagnostics to guide management.

MSD Ltd. has developed the Fungal Service Evaluation Tool (FSET), a secure database and analysis tool, to support UK clinicians managing patients at risk of breakthrough IFI (BIFI) to evaluate their antifungal management.

Aims: This service evaluation aimed to utilise the FSET to evaluate the impact of the antifungal management guidelines on healthcare resource utilisation associated with patients at risk of a BIFI.

Methods: An interim analysis of high-risk adult patients with prolonged neutropenia aged ≥18 years at initiation of antifungal prophylaxis/treatment was carried out. Retrospective data on patient characteristics, antifungal prophylaxis and treatment, IFI-related diagnostic tests, hospital attendance/admission during antifungal prophylaxis were collected for 12-month periods before and after 2014 (Cohort 1: 2013; Cohort 2: 2015). Anonymised data was entered into the FSET and this data was analysed using descriptive statistics.

Results: The evaluation included 24 patients in Cohort 1 (median age 66.8 [interquartile range (IQR): 47.5–72.2] years; 16 [67%] male; 5 [21%] ALL) and 22 patients in Cohort 2 (median age 66.8 [IQR: 51.7–73.4] years; 13 [59%] male; 1 [5%] ALL). At least one line of antifungal prophylaxis was recorded in 22 (92%) patients in Cohort 1 and 17 (71%) in Cohort 2. Posaconazole was the most commonly prescribed antifungal in Cohort 1 (18/24 [75%]) and Cohort 2 (17/22 [71%]). Other agents used included liposomal amphotericin B, fluconazole, and itraconazole. There were no patients in Cohort 1 and 2 (9%) patients in Cohort 2 (overall 4%) who experienced a BIFI: 1 was defined as confirmed and 1 as suspected. The mean 12 month costs per patient for all resource utilisation (including antifungal drug costs, hospitalisation costs [including admissions and attendances], investigations and tests) was £28,903 in Cohort 1 and £21,934 in Cohort 2 (Figure 1). Hospitalisation costs were a key determinant of overall costs, which is common in the management of people with complex underlying disease. There were 4 (17%) patients in Cohort 1 and 1 (5%) in Cohort 2 who had a period of ITU associated stay, which typically has greater costs than general wards. The most common investigations/tests were blood cultures (Cohort 1: mean 13.8; Cohort 2: mean 10.7) and chest x-ray (Cohort 1: mean 4.0; Cohort 2: mean 2.5), which are in-line with routine clinical practice. Once implemented, the guideline was adhered to in the management of 19 patients (86%) in Cohort 2.

Summary/Conclusions: These data show that rates of breakthrough IFI are low in complex patients receiving antifungal prophylaxis/treatment. Furthermore, the results in Cohort 2 indicate that the switch to recommending posaconazole tablets did not result in an increase in the mean cost per patient of antifungal prophylaxis and shows a lower overall mean cost per patient. A larger cohort study over a longer period is warranted to confirm these findings.
Iron metabolism, deficiency and overload

PB1901
REAL-LIFE FEASIBILITY OF AN IRON CHELATION PROGRAM WITH DEFERASIROX IN MYELODYSPLASIA AND OTHER ACQUIRED CHRONIC ANEMIAS: A SINGLE CENTRE EXPERIENCE

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Background: Prolonged red blood cell (RBC) transfusion support in patients affected by myelodysplastic syndrome (MDS) and other chronic anemias may cause vital organs damage due to accumulation of non-transferrin-bound iron with consequent increased oxidative stress. retrospective studies have shown that iron chelation may prevent aforementioned mechanisms and improve survival in low-risk MDS patients. Iron chelation is usually recommended in patients who received at least 20 RBC units and/or have a serum ferritin level of 1000 ng/ml or higher. Deferasirox, an oral iron chelator, has widely replaced the use of deferoxamine, due to its greater manageability, especially in the elderly. However, an high dropout rate of approximately 50% of patients within one year was observed in the majority of clinical studies, the leading cause of discontinuation being gastrointestinal (G.I.) and renal toxicity and skin rash.

Aims: We aimed at evaluating the real-life feasibility of a program of prolonged iron chelation in a population of acquired chronic anemia patients. Thus, we performed a retrospective analysis to evaluate which is the percentage of patients who in our center actually receive and tolerate deferasirox treatment, among the cohort of eligible patients.

Methods: Deferasirox treatment is considered at our centre in patients affected by MDS or other forms of chronic anemia (excluded chronic bleeding) who fulfill criteria for iron chelation (high transfusion burden, i.e. ≥20 RBC units and/or a serum ferritin ≥1000 ng/ml). Starting dose is usually 10 mg/kg, titrated up to 30-35 mg/kg as tolerated. The cohort of patients transfused at our centre during 2015 and 2016 was considered for analysis. Causes of unsuitability and of treatment discontinuation were extracted from our database.

Results: Our cohort consisted of 58 patients, mainly affected by MDS (45 pts); other diagnosis were myelofibrosis (6 pts), NHL (2) and multifactorial anemia, unrelated to chronic disease itself or to comorbodities, and pre-existing renal failure (4).

Overall, 25/58 (43%) patients had to interrupt the treatment, due to toxicity (mainly renal failure, followed by gastrointestinal toxicity), see flow-chart). Overall, 25/58 (43%) patients were assigned to iron chelation (see the Figure 1). The leading cause of ineligibility was gastrointestinal (5 pts), due to the hematologic disease itself or to comorbidities, and pre-existing renal failure (4).

Importantly, in 6 cases patients were not offered iron chelation without a specific clinical reason: half of them (3/6) were non-MDS patients. Furthermore, 13/58 patients had to interrupt the treatment, due to toxicity (mainly renal failure, followed by gastrointestinal toxicity, see flow-chart). Overall, 25/58 (43%) patients were assigned to iron chelation (see the Figure 1).

Summary/Conclusions: Together, our results demonstrate the feasibility of noninvasive transcutaneous spot-checking of total hemoglobin (SpHb) in children living in remote locations.

Methods: Transcutaneous noninvasive spot-checking of total hemoglobin (SpHb) was performed in children attending summer-school camps at 12 different locations in Cambodia. SpHb was measured in fingertips by using size adapted optic sensors. For the purpose of the study, three age groups were defined as follows: Group 1=less than 5 years, group 2=5 to 11 years, and group 3=11 to 14 years.

Results: A total of 476 otherwise healthy children were analyzed. Mean SpHb value was 11.9 ±0.93 g/dl (range 9-16 g/dl). Overall, the prevalence of anemia in the entire population was 34.5%. Anemia was present in 50/51 (16.1%) of the children within group 1, 97/189 (33.9%) in group 2, and 54/81 (40%) in group 3. (p=0.039, two sided Pearson’s Chi square). There were no differences in the prevalence of anemia by gender in groups 1 and 2. In group 3, anemia was significantly more prevalent in females 32/65 (49.2%) than in males 22/48 (31.4%), p=0.035.

Summary/Conclusions: Our results demonstrate the feasibility of noninvasive transcutaneous spot-checking of total hemoglobin (SpHb) for the screening of anemia in children from remote rural areas with limited access to health services. Our results also confirm the high prevalence of anemia in this population.

PB1903
IRON DEFICIENCY ANEMIA IN INFANTS AND YOUNG CHILDREN

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Background: Iron deficiency in infants and young children is easy to be underdiagnosed. Anemia and iron deficiency are usually corrected after aged 2-3 years old, but it causes complications. There is an association between IDA and impaired neurocognitive function and exercise intolerance, even after treatment of IDA. Therefore, preventing the progression of iron deficiency is especially important during infancy and early childhood. When increased vulnerability is associated with rapid growth and development, especially of the brain.

Aims: To detect iron deficiency anemia early and to reduce the adverse impact by IDA, we assessed the characteristics of infants and young children with IDA, those at risk for IDA and those exhibiting associated characteristics of severe anemia.

Methods: Among 1,782 children with IDA aged 6 months to 18 years-old, we retrospectively analyzed medical records and laboratory data of 1,361 subjects aged 6–23 months with IDA who had been diagnosed between 1996 and 2013. We excluded patients with CRP ≥5 mg/dL.

Results: IDA was predominant in boys (214:1) during infancy and young childhood. Peak incidence was at 9 to 12 months of age. Only 7% of subjects were brought to the hospital with symptoms and/or signs of IDA, while 23.6% in subjects with severe IDA. LBW infants with IDA shows low adherence with iron supplementation. In a multivariate analysis, risk factors of severe anemia in infants included prolonged breastfeeding without iron fortification (odds ratio (OR) 5.70) and low birth weight (OR 6.49).

Summary/Conclusions: Many clinicians did not consider IDA as a real problem, so many children with IDA were not followed up. LBW infants need more attention. Increase adherence of iron supplementation. For early detection of IDA, nutritional assessment should be evaluated in every infants and iron batteries in high risk infants (LBW infants, prolonged breastfeeding, pica eater and/or symptoms of IDA) at health screening visit.
PB1904

THE ROLE OF ZINC PROTOPORPHYRIN IN THE DIAGNOSIS OF SIDEROPENIC ANEMIA

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Background: Sideropenic anemia (IDA) is the main cause of anemia worldwide. Even though, its diagnosis is quite straightforward with the use of red blood cells indices, peripheral blood smear (PBS) and ferritin measurements, there are still some pitfalls, namely in the presence of inflammation. The chelation of iron by protoporphyrin constitutes the final reaction of heme biosynthesis. In the absence of iron, zinc becomes an alternative substrate for ferrochelatase leading to the formation of zinc protoporphyrin (ZPP). This compound can be quantified by fluorometry in blood samples, proving itself as a useful and easy parameter for the diagnosis of IDA. However, this technique is not broadly used in the clinical practice.

Aims: Determine the cut-off value of ZPP for the diagnosis of IDA. Evaluate the value of ZPP for the differential diagnosis between IDA and anemia due to inflammatory diseases (AID).

Methods: We have analyzed in our lab, from 1st to 15th February 2017, all the complete blood counts (pediatric and adult) with anemia (as defined by WHO) which had sedimentation rate (SR) and serum ferritin evaluations.

We have defined three different groups: IDA: Anemia and Ferritin <20µg/L; AID: Anemia, Ferritin >20µg/L and SR<20mm/h; Group control (GC): Normal levels of Hb adjusted by age and sex, as defined by WHO, Ferritin 20-120µg/L and SR<20mm/h. ZPP measurement was performed by hematobiumetry (AIV, Biomedica, Inc.). Data were analyzed by SPSS v20.0 using Wilcoxon W and Man-Whitney to examine differences between groups and receiver-operating characteristic (ROC) analysis to determine the cut-off values of ZPP. We consider values of cells and ZPP statistcally significant a p-value <0.05.

Results: We have identified 204 samples that fulfilled the inclusion criteria: 104 with IDA, 51 with AID and 49 from control patients. IDA group: 73% female (F); mean age 32.3 y in F [1.1-78], 28 y in males (M) [1-78]; mean Hb was 10.6g/dL [SD 1.4]; mean ferritin was 9.3 µg/L [SD 4.85] and ZPP was 214.1 µmol [SD 121.3]; mean SR was 20.0 mm/h [SD12.9]; AID group: 75% F; mean age 47 y [2-91] and 22y in M [1-85]; mean Hb 11.0 g/dL [SD 1.2]; mean ferritin 150.3 µg/L[SD246.2] and ZPP 136.7 µmol [SD 107.8]; mean SR 47mm/h [SD 4]. The mean serum ZPP in IDA and AID was significantly higher than in GC (95% CI; p<0.0005). The ROC analysis showed 83.7% sensitivity and 85% of specificity to identify IDA for ZPP ≥100.3 µmol (Wt=0.933) and 69% sensitivity and 70% of specificity to identify AID for ZPP >140 µmol (Wt=0.749) when compared with GC.

Summary/Conclusions: We have concluded that ZPP is a valid, quick, easy and cheap parameter to diagnose IDA in clinical practice, and we have defined in our cohort of patients a ZPP cut off of ≥100.3µmol as diagnostic of IDA with 83.7% sensitivity and 85% of specificity, independent of age. In AID patients we found a cut-off value of ≥140µmol, but with a low sensitivity and specificity. In clinic study ZPP was not a reliable method to differentiate IDA from AID. This could be due to a sample selection bias (since clinical data were missing and the number of patient with AID was substantially lower than with IDA). It would be important to enlarge the AID sample in order to obtain a more reliable result. Since the measurement can be performed in capillary blood and it is a very quick and cheap method to diagnose IDA, this could be a powerful tool in under-developed countries.

PB1905

HYPERFERRITINEMIA AND SERUM INFLAMMATORY CYTOKINES IN ADULTS WITH NEWLY DIAGNOSED HEMOGLOPHIC LYMPHOHISTIOCYTOSIS ASSOCIATED WITH HEMATOLOGICAL MALIGNANCY

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Background: Hemophagocytic lymphohistiocytosis (HLH) is an underdiagnosed but life-threatening syndrome of hyperinflammation which in adults is often caused by hematological malignancies. Release of inflammatory cytokines in HLH induces cell death and cytokine production that cumulates in cytokine storm and hyperinflammation. Hyperferritinemia ≥500 µg/L is a diagnostic criterion for HLH. Prevalence of hyperferritinemia in HLH in the adult population is much less established than in children.

Aims: The aim of the present study was to evaluate the frequency and extent of hyperferritinemia as well as serum concentrations of selected inflammatory cytokines at the time of diagnosis of hematological malignancy-associated HLH (HM-HLH) in adults.

Methods: The study included 71 adults with HM-HLH, aged 22–84 years, and diagnosed between 2009 and 2016. Hematological malignancy was defined as a neoplasm of lymphoid or myeloid origin. In all studied patients, the diagnosis of HLH was based on the HLH-2004 criteria. Since the majority of patients in this study had severe lymphopenia, we decided to not perform functional analyses of NK-cells for HLH diagnosis. Thus, we included in this analysis all patients with hematological malignancies and suspected HLH who fulfilled at least four of seven HLH-2004 criteria as well as at least two of three additional features: sIL-2Rα ≥2400 U/mL, hemophagocytosis in BM, and hyperferritinemia ≥10,000 µg/L. Serum concentrations of inflammatory cytokines IL-1β, IL-6, IL-8, IL-10 and TNF-α were analyzed using chemiluminescence (IMMULITE®1000 Immunoassay System (DPC Siemens).

Results: Lymphoid malignancy was diagnosed in 42 patients and myeloid malignancy in 29 patients. Fifty-four (76%) patients developed HLH as a first clinical manifestation of an unknown malignancy, during progressive disease, or at malignancy relapse. The remaining 24% of patients developed HLH during chemotherapy. Serum ferritin concentration (ref.: 30–350 µg/L) at the time of HM-HLH diagnosis was elevated in all but one patient (707, 98%). Mean ferritinemia was 37.28±84.44 µg/L, median value 14.727 µg/L, and ferritinemia range 96–645,291 µg/L. As HLH-2004 criteria, hyperferritinemia ≥500 µg/L was present in 69 of 71 patients (97%) at the time of HLH diagnosis. Hyperferritinemia of ≥2000 µg/L was noted in 67 (94%) patients, hyperferritinemia of ≥5000 µg/L in 56 (76%) patients, and hyperferritinemia of ≥10,000 µg/L occurred in 42 (59%) patients. Serum levels of sIL-2Rα (sCD25) were measured in 69/71 patients, of which 91% (63/69) had values ≥2400 U/mL. Moreover, in 5 more patients sIL-2Rα was clearly elevated to 2179, 2233, and 2345 U/mL, respectively. Concentrations of TNF-α, IL-6, and IL-10 in serum were in each patient in over 85% of the examined hM-HLH patients. IL-8 concentration was increased in half of all tested patients at the time of HLH diagnosis. However, IL-1β concentration was above reference range only in 12% of patients (7 of 58). Results of the inflammatory cytokine analyses in patients with newly diagnosed HM-HLH are presented in Table 1.

Table 1. Inflammatory cytokines in patients with newly diagnosed HM-HLH.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Normal Range</th>
<th>Mean ± SD</th>
<th>Median</th>
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<td>IL-1β</td>
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<td>IL-8</td>
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Summary/Conclusions: Hyperferritinemia at the time of HLH diagnosis was common in Swedish adult patients with HM-HLH. Hyperferritinemia ≥500 µg/L was present in vast majority (97%) of them. We would like to emphasize that serum ferritin level fluctuates and can differ significantly from one day to another. Ferritinemia should be repeatedly measured in cases of suspected HLH. Serum concentrations of TNF-α, IL-6, IL-8, and IL-10 were frequently elevated in the examined HM-HLH patients and these can become important markers supporting HLH diagnosis in equivocal cases. On the other hand, IL-1β seems to be less useful in confirming a cytokine storm in this patient group.

PB1906

REDUCING UNNECESSARY BLOOD FILMS USING AN IRON DEFICIENCY ALGORITHM

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Background: In 2015, Wellington SCL (WSCL) was selected to provide integrated laboratory services in Wellington region, New Zealand (NZ). This involved merging services from previous community laboratory Aotea Pathology Ltd. (APL) with the three regional hospital based District Health Boards (DHB) laboratories - Capital & Coast (CCDHB), Hutt Valley and Wairarapa. On the 1st of November 2015, WSCL would launch its new integrated service with a merged workforce, new technology, processes and procedures. Considered to be the biggest laboratory integration project undertaken in NZ, every effort needed to be made to reduce the workload without compromising patient care.

Aims: In the Haematology laboratory, one of the most common triggers for
were estimated using Toshiba chemical analyzer (Toshiba, Japan).

Methods: An algorithm was designed in IT3000 to encourage testing and treatment for iron deficiency using a series of automated educational comments, while minimising unnecessary laboratory work. The impact that this algorithm had at WSLC was investigated by retrospective analysis of all the patient results from 1st November 2015 to the 1st of May 2016.

Results: In the first six months of operation, WSLC performed 232,192 CBCs and 30,204 blood films with an average review rate of 13.01%. Had this algorithm not been employed, 2,434 extra blood films would have been reviewed, bringing the review rate up to 14.05%.

Summary/Conclusions: Incorporation of an algorithm specific for iron deficiency in IT3000 has significantly reduced the review rate without any negative impact on patient care.

PB1907
THE RELATIONSHIP ENDOTHELIAL MICROPARTICLES AND ASYMMETRIC DIMETIL ARGinine IN CHILDREN WITH IRON DEFICIENCY AND IRON DEFICIENCY ANEMIA
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Background: Iron deficiency anemia and iron deficiency without anemia increase the risk of atherosclerosis by increasing oxidative stress and inflammation. Endothelial dysfunction is an important factor of the pathogenesis of atherosclerosis.

Aims: Endothelial micro particles (EMPs) are considered as markers of endothelial dysfunction. Asymmetric dimetil arginine (ADMA) is known as another marker of endothelial dysfunction. In this study: we aimed to evaluate circulating EMPs and ADMA in children with iron deficiency and iron deficiency anemia and to disclose iron deficiency with the strongest relation with EMPs, ADMA and carotid atherosclerosis.

Methods: This study included 30 children with iron deficiency anemia, 30 children with iron deficiency without anemia and 30 healthy children whose anthropometrics measurements were recorded. Hemoglobin, serum iron level, iron binding capacity, ferritin, and lipid profile were studied. Circulating EMPs (CD144, CD146, and CD105) were measured by flow cytometry. ADMA was measured by ELISA. The carotid artery intima media thickness (CIMT) and left ventricular mass index (LVMI) were measured using echocardiography.

Results: CD144 and CD105 EMP levels were lower in the iron deficiency without anemia group than in the control group and statically lower than in the iron deficiency anemia group (p<0.05). There were no significant differences in ADMA level between groups. Any significant variety in ADMA level was not observed between groups. CIMT was negative correlated with ferritin and high density lipoprotein and positive correlated with body weight.

Summary/Conclusions: In this study, endothelial dysfunction which occurs as a result of iron deficiency were observed. According to our result, CD144 and CD105 EMP levels in the iron deficiency without anemia group were lower than the iron deficiency anemia and control group; these levels in iron deficiency anemia group were higher than control group. In addition, when the level of ferritin has decreased, CIMT has increased. This study show that CD144 and CD105 may be related to endothelial dysfunction which occurs by iron deficiency.

PB1908
INVESTIGATION OF IRON METABOLISM FOR REGULATING MEGAKARYOPOIESIS AND PLATELET COUNT ACCORDING TO THE MECHANISMS OF ANEMIA
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Background: Iron deficiency anemia (IDA) is characterized by depletion of total body iron stores. By contrast, chronic inflammation makes iron unavailable for hematopoiesis through a cytokine-mediated cascade, resulting in anemia of chronic disease (AOC). However, the laboratory data regarding the regulatory role of iron metabolism on platelet count has not been fully discussed yet.

Aims: In this study, we investigated the relationship between iron status and platelet production according to different anemic mechanisms representing different iron metabolisms.

Methods: The study included total of 759 blood specimens from 537 different patients. The complete blood count with various CBC index were measured using Advia 2120 (Siemens, USA). Biochemical indexes including iron level were estimated using Toshiba chemical analyzer (Toshiba, Japan).

Results: We found a significant relationship between platelet count and serum iron level in AOC group (p=0.27), whereas there was no correlation in IDA group. In AOC group, platelet count was significantly correlated to serum iron level only in AOC group with decreased serum iron level (p<0.0001), unlike AOC group with normal serum iron level.

Summary/Conclusions: Reactive thrombocytosis in inflammatory states may alter the production of platelets in AOC. Moreover, iron deficiency in AOC involves upregulated hepcidin production induced by increased inflammatory cytokines. It can cause increased iron sequestration in macrophage and decreased iron absorption for bone marrow. The condition of decreased megakaryocytic iron supply makes megakaryocytes with higher ploidy which can release more platelets than lower ploidy. These two features may enhance thrombocytosis in patients of AOC with decreased iron level. In the future, the further study should be performed to elucidate underling mechanism involving the tight regulation between iron metabolism and megakaryopoiesis in anemic patients.
Myelodysplastic syndromes - Biology

PB1910

ROLE OF PRO-PHAGOCYTIC CALREICULIN AND ANTI-PHAGOCYTIC CD47 IN MDS AND MPN MODELS TREATED WITH AZACYTIDINE OR RUXOLITINIB

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Background: Myelodysplastic syndrome (MDS) and Myeloproliferative neoplasms (MPN) are clonal myeloid disorders with the tendency to progress into acute myeloid leukaemia. Previous studies in solid tumours have shown an increase in expression of both pro-phagocytic calreticulin (CALR) and anti-phagocytic CD47, as they act in response to one another, reflecting a possible apoptosis vs survival mechanism in response to chemotherapy.

Aims: The aim of our study is to assess the changes in CALR and CD47 levels during treatment of MDS and MPN with azacytidine (AZA) or ruxolitinib (RUXO), in a series of model cell systems.

Methods: CALR and CD47 gene and protein expression was measured in MDS cell line models (MOLM-13 and SKM-1), MPN cell line models (HEL-92 and GDM-1) and in an intermediate MDS/MPN cell line (K562) before and after treatment with AZA and RUXO. Drug titrations were completed, resulting in dosing regimens of 0.05μM/ml for both AZA and RUXO, with re-drugging occurring at 24 hours. Cells were then harvested, cDNA was synthesized for use in qPCR and protein levels determined by Western blot analysis.

Results: When treated with AZA, MDS cell models showed a 7-10 fold increase in CALR expression and 4-6 fold increase in CD47 expression. In contrast, the MDS/MPN intermediate model cell (K562) showed a 4.5 fold increase in CALR but only a 0.5 fold increase in CD47 expression. In the MPN model HEL-92, a 4 fold increase in CALR and 4.6 fold increase in CD47, was observed, whereas in the other MPN model (GDM-1 cells) expression was more evenly matched between CALR and CD47 (5.3 and 4.8 fold increases, respectively). After treatment with RUXO, MPN models showed a 9.5-16 fold increase in CALR expression and a 6-9 fold increase in CD47, which would be expected as RUXO is used to treat MPN in humans. When the MDS/MPN cell model or pure MDS models were treated with RUXO, the ratio of CALR/CD47 decreased substantially (with CALR expression only increased 2.4-3.7 fold compared to CD47 increasing 4.6-6.9 fold) showing resistance to treatment and a significant anti-phagocytic response. Interestingly one of the MDS cell line models (MOLM-13) showed an unexpectedly good response to RUXO therapy with high CALR/CD47 ratio (8 fold vs 4.8 fold, respectively).

Summary/Conclusions: In line with results in solid tumours, we have shown that treatment for MDS and MPN leads to an up-regulation of CALR and, to a lesser extent, CD47 in cell line models. The ratio of CALR/CD47 seems to correlate with specific treatment response, significantly increasing when given diseases models are treated with the appropriate drug. We postulate a role of CALR expression in leukaemia cell phagocytosis, with CD47 co-expression in synergy as a protective instinct within the cell to try and prevent apoptosis. Some MDS models showed an excessive rise in CD47 expression and low expression of CALR. This indicates that the CD47 mediated anti-phagocytosis takes control and suppresses the CALR expression, leading to cancer cell survival and ineffectiveness of treatment. These results need to be validated in human samples at different stages of disease to allow a better understanding of treatment response and/or resistance to chemotherapy within these diseases.

PB1911

GENETIC VARIANTS OF MSH3 AND BLM GENES MAY INFLUENCE MYELODYSLASTIC SYNDROME SUSCEPTIBILITY AND PROGNOSIS

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Background: Myelodysplastic syndrome (MDS) is a heterogeneous group of hematopoietic stem cell disorders, characterized by peripheral cytopenias, ineffective hematopoiesis and frequent transformation into acute myeloid leukemia (AML). Segregation studies in MDS patients have shown deletions of chromosome 5q in patients with 5q- MDS. The 5q- syndrome has a constant macrocytic anemia and normal or high platelet counts associated with hypolobulated megakaryocytes. Previous studies have detected reduced RPS14 expression in more than 50% of non-5q- patients. Recently, the pivotal role of RPS14 in human erythropoiesis during 5q- MDS pathology has been demonstrated; RPS14 haploinsufficiency produces the activation of p53 and its target p21 in erythroid cells, resulting in cell cycle arrest and apoptosis. Based on these results, non-5q- patients expressing low levels of RPS14 will be potentially benefitted by lenalidomide therapy. In this work, we explore the origin of the reduced RPS14 expression in non-5q- patients and its potential link with 5q-pathology.

Aims: The objective of this work was to explore the origin of RPS14 low expression in non-5q- MDS patients and its link with 5q-pathology. In order to do this, we analysed potential mutations in RPS14 gene. We also studied expression changes in other key genes involved in the development of the 5q- disease, including the tumour suppressor gene SPARC and the putative tumour suppressor gene CSNK1A1, contained in the commonly deleted region. Moreover, other 32 genes related with MDS disorders were evaluated in relation with RPS14 levels. Finally, in order to establish if this group of patients could be benefitted by lenalidomide therapy, p21 expression levels were also analysed.

Methods: DNA and RNA were extracted from the bone marrow of 89 non-5q- MDS patients. Ten controls and nine 5q- MDS patients were used as negative and positive controls, respectively. RPS14, SPARC, CSNK1A1 and p21 mRNA levels were analysed by real time PCR using Taqman probes and a 7500 RT PCR System. β-glucuronidase gene was used as endogenous reference to normalize data. Samples were classified by RPS14 expression levels and differences in SPARC, CSNK1A1 and p21 expression mean values between the two groups were analysed using the Mann-Whitney U test. RPS14 and 32 genes expression were also analysed using Ion Proton sequencing.

Results: Non-5q- patients expressing low levels of RPS14 presented higher survival probability in the IPSS lower risk group. This data, in addition with a tendency for increased p21 expression, suggests that this group could be benefitted by lenalidomide therapy. Nevertheless, we did not observe a significant interaction between RPS14 expression and the presence of other high risk features in these patients. In summary, our results suggest that changes in RPS14, discarding alterations in the adjacent genes commonly deleted in 5q- MDS patients. In addition, the majority of patients analysed did not present any mutation in RPS14 gene. Only two MDS patients showed mutations upstream, downstream or within intronic regions of the gene. The origin of RPS14 expression alteration in these patients have to be confirmed.

Summary/Conclusions: Although the important role of RPS14 in MDS pathol-
ogy has been recently demonstrated, the origin of RPS14 downregulation in more than 50% of non-SLD patients remains unknown. Our results suggest that the origin of RPS14 decreased expression is not related with genomic alterations in 5q region. Further studies are necessary in order to establish a link with 5q-pathology and demonstrate the potential use of lenalidomide in this group of patients.

Background: A prospective study was performed over one year in order to investigate whether suspected myelodysplastic syndromes (MDS) could be detected on a complete blood counts (CBC), the fastest laboratory investigation, performed on the recently developed XN-10® (Sysmex, Kobe, Japan).

Aims: The primary end point was to discriminate MDS patients from normal samples and the secondary end-point was to distinguish MDS with excess blasts (MDS-EB), MDS with multilineage dysplasia (MDS-MLD), MDS with single lineage dysplasia (MDS-SLD) and MDS with ring sideroblasts and sideroblasts within the MDS group and by comparison with controls as described by the WHO 2016 classification.

Methods: One hundred and thirteen patients were enrolled in the study, for whom a diagnosis of MDS was concluded based on CBC, bone marrow smears examination and karyotype. All patients were free of treatment, including transfusions, at inclusion. They were 63 men and 50 women with a median age of 82 years (range 36-96 yo). CBC were performed on a Sysmex analyzer XN-10® including classical parameters (hemoglobin level, Mean Corpuscular Volume (MCV), reticulocytes, platelets, neutrophils and extra-parameters i.e. platelets by fluorescene (PLT-F), immature platelets fraction (IPF%), immature reticulocyte fraction (IRF%) and the neutrophils median position of the three axes as well as their dispersion (Neut-WX). For comparison with normal values, results from 707 healthy subjects over 50 years old, for whom CBC were performed on the same analyzer and generated no flag, were used. All had parameters within the normal range according to age. According to the WHO, 37 patients were categorized in the MDS-SLD group, 35 in MDS-MLD, 24 in MDS-EB, 35 in MDS-RS-SLD within the MDS group and by comparison with controls as described by the WHO 2016 classification.

Results: Both classical and extra parameters indeed showed significant differences between the subgroups tested. Among the whole group of MDS patients a number of parameters of all lineages were statistically different from the healthy cohort. The median level of hemoglobin was 9.92±1.96 g/dL (p<0.0001), the median MCV (99.24±10.56 fL p<0.0001), reticulocyte counts 44.3x10⁹/L (range 18-163, p<0.0001), 16.7% (range 2.4-50.0%, p<0.0001). The median platelet count was 194 ±128x10⁹/L (p<0.0001) and the median IRF% 8.8% (1.2-42; p<0.0001). Among leukocyte parameters, the MDS median neutrophil count was significantly lower at 3.08±2.58x10⁹/L (p<0.001) while Neut-WX was significantly higher (367±1; p<0.0001). The latter, allowed to predict a diagnosis of MDS with 73% sensitivity and 97% specificity. For patients over 50 years old, 4 parameters (Neut, Neut-WX, hemoglobin level and MCV) in a score allow to diagnose MDS with 92% sensitivity and 81% specificity. When considering MDS subgroups, although each of them was significantly different from controls for hemoglobin levels, MCV and IRF% and (p<0.001), they could not be discriminated with the score of patients with MDS with single lineage dysplasia and ring sideroblasts, platelets were similar to those of controls, yet significantly higher than for MD-EB or MDS-MLD (p=0.004 and p=0.029 respectively).

Moreover, neutrophils counts were significantly lower in MDS-DML or MDS-EB than in MDS-SLD-R.

Summary/Conclusions: This study demonstrates that a simple CBC allows to screen for MDS using a multiparameter score including Neut-WX. Blood smear examination should be performed in this situation even if the XN-10® analyzer does not raise any alarm, especially in unknown patients older than 50.

PB1915

CORRELATION OF PATIENT PROGNOSIS WITH PU.1 AND JDP2 PROVIDES POTENTIAL NOVEL PROGNOSTIC/DIAGNOSTIC MARKERS IN MDS

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Background: PU.1 is a key transcription factor in haematopoiesis that plays important roles in various haematological malignancies. Previously, significant down-regulation of PU.1 has been reported in high risk myelodysplastic syndromes (MDS) and acute myelogenous leukemia (AML) patients.

Aims: Here we investigated PU.1 motif binding function in 35 MDS patients, including 14 with myelodysplastic syndromes (MDS), 7 with AML, 1 patient with RAEB-1 and another with CMMML. These patients had a higher% of ring sideroblasts and blasts; higher scores on IPSS, IPSS-R and WPSS; lower platelet counts, higher erythropoietin levels and greater expression of CD34 / CD117 / CD123 / GlicoP / IL-6 / TNFα and molecular characteristics (methylation profile of genes p15, p16, DAPK, R1, R2, R3 and R4 performed by PCR-MS, and evaluation of expression levels of regulatory molecules of apoptosis BCL-2, BAX, TRAIL, R1, R2, R3, FAS, Survivin, Casapte 3, Cit C, Glycop and p53, by FACS).

Results: In the 60-month follow-up, 11 patients progressed to Acute Myeloblastic Leukemia (AML), 7 with RAEB-2, 2 with RCM1, 1 patient with RAEB-1 and another with CMMML. These patients had a higher% of ring sideroblasts and blasts; higher scores on IPSS, IPSS-R and WPSS; lower platelet counts, higher erythropoietin levels and greater expression of CD34 / CD117 / IL-6. Assigning a value (+1) to each altered variable a new prognostic score was obtained, which we named Progression Score for Acute Leukemia. We observed that patients belonging to subtypes with the highest scores were those that progressed to AML, namely RAEB-1, RAEB-2 and RCM.

Summary/Conclusions: In conclusion, we believe that this score may contribute to evaluate the risk of progression to AML, by reflecting the heterogeneity of MDS and its multifactorial etiology. The coexistence of many altered variables not only contributes to the etiopathogenesis of MDS but also allows the assessment of potential leukemic transformation.

PB1914

PROGRESSION SCORE FOR ACUTE LEUKEMIA – A NEW PROGNOSTIC SCORE IN MDS

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Background: Since 1997, the International Prognostic Scoring System (IPSS) has been the standard for stratifying patients with Myelodysplastic Syndrome (MDS). Although other models were proposed to improve this stratification, some issues remain, notably the identification of low-risk patients with poor prognosis who may benefit from earlier and/or aggressive therapy.

Aims: The aim of our work was the conception of a new prognostic score in MDS, based in the cellular and molecular disease characteristic.

Methods: Our sample consisted of 102 patients diagnosed with MDS de novo. The median age was 74 years (22-89), with a 0.8 Male to Female ratio. The subtypes, according to the World Health Organization 2008, were Refractory Cytophenia with Multilineage Dysplasia (RCMD) (n=52), Refractory Cytophenia with Unilineage Dysplasia (RCUD) (n=12), Refractory Anemia with Excess Blasts type 1(RAEB-1) (n=8), RAEB-2 (n=6), Refractory Anemia with Ringed Sideroblasts (n=6), 5q- syndrome (n=4) and Chronic Myelomonocytic Leukemia (n=12). The IPSS based stratification was: low (n=37), intermedi-1 (n=39), intermediate-2 (n=10) and high (n=1). Several variables were evaluated: hematological (hemoglobin, platelets, blasts and ring sideroblasts), biochemical (erythropoietin, β2-microglobulin, folic acid, vitamin B12, ferritin, LDH), immunophenotypic (hematopoietic stem cell characterization by flow cytometry, FC, using the markers, CD34 / CD117 / CD123 / GlicoP / IL-6 / TNFα) and molecular characteristics (methylation profile of genes p15, p16, DAPK, R1, R2, R3 and R4 performed by PCR-MS, and evaluation of expression levels of regulatory molecules of apoptosis BCL-2, BAX, TRAIL, R1, R2, R3, FAS, Survivin, Casapte 3, Cit C, Glycop and p53, by FACS).

Results: In the 60-month follow-up, 11 patients progressed to Acute Myeloblastic Leukemia (AML), 7 with RAEB-2, 2 with RCM1, 1 patient with RAEB-1 and another with CMMML. These patients had a higher% of ring sideroblasts and blasts; higher scores on IPSS, IPSS-R and WPSS; lower platelet counts, higher erythropoietin levels and greater expression of CD34 / CD117 / IL-6. Assigning a value (+1) to each altered variable a new prognostic score was obtained, which we named Progression Score for Acute Leukemia. We observed that patients belonging to subtypes with the highest scores were those that progressed to AML, namely RAEB-1, RAEB-2 and RCM.

Summary/Conclusions: In conclusion, we believe that this score may contribute to evaluate the risk of progression to AML, by reflecting the heterogeneity of MDS and its multifactorial etiology. The coexistence of many altered variables not only contributes to the etiopathogenesis of MDS but also allows the assessment of potential leukemic transformation.
PB1916
DECREASED EXPRESSION OF DECORIN, A WNT-PATHWAY RELATED PROTEIN, IN MESENCHYMAL STEM CELLS OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES
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Background: Myelodysplastic syndromes (MDS) are clonal disorders of the haematopoietic stem cells (HSCs) characterized by inefficient bone marrow (BM) haemopoiesis and increased risk for leukemic evolution. Ineffective BM haemopoiesis in MDS has also been linked with an abnormal microenvironment that may sustain or even induce the aberrations within the HSC compartment. We have previously shown that the stroma progenitor cells, namely the mesenchymal stem cells (MSC), in MDS patients display impaired clonogenic and proliferative potential, reduced haemopoiesis supportive capacity and downregulation of the canonical Wnt-signaling pathway.

Aims: Decorin, a small leucine-rich proteoglycan, and galectin-3, a member of b-galactosidase specific lectin family, are components of the extracellular matrix of the BM microenvironment. Both proteins have been implicated in the canonical Wnt-pathway participating therefore in cell growth and proliferation. The aim of the study is to assess the expression of decorin and galectin-3 in MSCs of MDS patients, evaluating their implication in the abnormal Wnt-signaling previously reported in MDS.

Methods: BM MSCs were isolated from 12 patients with lower risk MDS aged 51 to 75 years (median 67.5 years) and 12 haematologically healthy subjects aged 50 to 73 years (median 63.3 years), after informed consent. The study has been approved by the Ethics Committee of the University Hospital of Heraklion. BM MSCs were characterized according to international system for human cytogenetic nomenclature (ISCN) criteria, expanded and re-seeded for two passages (P). Total RNA was extracted from culture-expanded P2 MSCs and amplified by real-time PCR for the evaluation of decorin and galectin-3. Relative gene expression was calculated by the ΔCT method.

Results: A statistically significant decreased expression of decorin was identified in MSC of MDS patients (mean 1.338, SD 0.84) compared to the healthy individuals (mean 1.830, SD 0.71). (P<0.05). Galectin-3 expression was also decreased in MDS patients (mean 0.6758, SD 0.50) compared to controls (0.9395, SD 0.50), although not at a statistically significant levels.

Summary/Conclusions: MSCs from MDS patients display statistically significant decreased expression of decorin and a tendency towards decreased expression of galectin-3 in BM MSCs compared to healthy individuals. These preliminary data indicate that extracellular matrix proteins may have a role in the disturbed Wnt-pathway signaling and abnormal MSC function in MDS patients. The underlying mechanisms are currently under investigation.

PB1917
CLINICAL FEATURES, CYTOGENETIC STUDY AND OUTCOME OF ADULT MYELODYSPLASTIC SYNDROMES: REVIEW OF 101 CASES, A SINGLE CENTER EXPERIENCE IN ALGERIA
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Background: Myelodysplastic syndromes (MDS) are heterogeneous disorders defined as clonal diseases involving hematopoietic stem cells and even characterized by cytopenias, with a high risk of leukemic transformation. Morphological analysis of peripheral blood (PB) and marrow aspirates or bone marrow biopsies is the first step that ensures a diagnosis of MDS. Cytogenetic studies are important means of defining different prognostic groups and even of showing how patients are eligible for this or that treatment. We conducted the first study including conventional karyotyping and fluorescent in situ hybridization (FISH) for MDS in our country.

Aims: Our study was aimed to evaluate outcome of MDS regarding IPSS and IPSS-R classification in an emerging country.

Methods: Between January 2012 to December 2016, 101 patients with MDS were consecutively diagnosed. Frequent genetic abnormalities in MDS were screened by R-banding karyotype and metaphasic and interphasic FISH using a panel including six probes (5q-,7q-,20q-, del(17p13), MLL, Inv(3) t(3;3). Between January 2012 to December 2016, 101 patients with MDS were consecutively diagnosed. Frequent genetic abnormalities in MDS were screened by R-banding karyotype and metaphasic and interphasic FISH using a panel including six probes (5q-,7q-,20q-, del(17p13), MLL, Inv(3) t(3;3).

Results: Among these 101 pts, 58 were male with a sex ratio=1.35; range in age is from 18 years to 94 years with a median of 61, 6 years. Median hemoglobin level was 8.80 g/dL (29-150), more than 60% of patients had severe anemia mainly in the low risk group; the median absolute neutrophil count was 3 G/L (0.060-13.5), and the median platelet count was 144 G/L (5-659). Median bone marrow blast value was 4% (0-18). Cases were classed by cytology morphometry FAB as RA (n=45), REAB (n=34), RARS (n=16), other (n=6). Classification by WHO was 47 cases MDS-EB1 (n=2), MDS-EB2 (n=3), MDS-EB3 (n=2), MDS-EB4 (n=1), RAEB-1 (n=22), RAEB-2 (n=13), RARS (n=15), isolated 5q (n=4). Among 101 patients, cytogenetic abnormalities by R banding karyotype (n=84) and FISH (n=101) were found in 41 cases (41%) distributed as single anoma-
Myelodysplastic syndromes - Clinical

PB1918

CLINICAL EVOLUTION OF ACUTE MYELOID LEUKEMIA WITH MYELODYSPLASIA-RELATED CHANGES
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Background: Acute myeloid leukemia (AML) with myelodysplasia-related changes (MRC) is usually classified associated to worse clinical course and poor prognosis compared other AML subtypes. Differences between treatment modalities according to age, and the response to treatment, would help to provide specific anti-AML treatment in this difficult scenario.

Aims: The objective of this study is analyze the clinical features and course of patients with AML with MRC, in order to evaluate the impact of different therapeutic regimens in this subgroup.

Methods: We report an unicentric retrospective study of 76 patients with AML with MRC, over the past ten years in a single institution in Spain. We analyzed the overall survival (OS) among the subgroup of patients with over or under 65 years, and the different types of treatment that has been offered.

Results: Median age was 69 years with a male predominance, and 66% was preceded by a known myelodysplastic syndrome with a median interval of 18 months to progress to AML. The more frequent genetic abnormalities in descending order were trisomies, del(5q), and del(7q)-. The patients aged >65 and <65 were 70% and 30%, respectively. The patients aged >65 received induction chemotherapy with anthracycline-cytarabine combinations so as to continue with post-consolidation management with allogeneic transplantation, but the 44% died over the induction chemotherapy (OS: 2.2 months). The OS in patients aged <65 was 20.2 months in chemotherapy plus allogeneic transplantation. The OS in patients aged >65 was 10.3 months in the group of anthracycline-cytarabine combinations, 3.81 months in chemotherapy plus allogenic transplantation, and 0.5 months in supportive measures group (Figure 1).

Summary/Conclusions: The AML with MRC patients is a group with difficult treatment decisions and tumor progression, in whom only the chemotherapy plus allogeneic transplantation treatment manage long-term survival. In patients aged >65, there is not a significant difference among groups, although the chemotherapy with anthracycline-cytarabine seems to reach a better OS versus other available treatments.

PB1919

SAFETY, EFFICACY, AND PHARMACOKINETICS OF INTRAVENOUS RIGOSERTIB IN JAPANESE PATIENTS WITH RECURRENT/RELAPSED OR REFRACTORY MYELODYSPLASTIC SYNDROMES: A MULTICENTER, OPEN-LABEL, PHASE I STUDY
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Background: Rigosertib, a novel phosphoinositide 3/4-kinase kinase inhibitor, selectively induces the apoptosis of cancer cells and is safe and well tolerated in pts with recurrent/relapsed or refractory MDS.

Aims: We conducted a multicenter, open-label, Phase I study of intravenous rigosertib to evaluate its safety, efficacy, and pharmacokinetics and to determine the recommended dose (RD) for Japanese pts.

Methods: The key eligibility criteria were as follows: recurrent/relapsed or refractory MDS; age: 20 or older; FAB classification (RA, RARS, RAEB, RAEB-1, and CMML), excepting patients at IPSS low- or Int-1-risk with respect to RA; ECOG PS of 0 to 2; no major organ dysfunction; and written informed consent.

Between June 2012 and February 2015, 7 male and 2 female pts (median age: 70; range: 63-84) were enrolled, and 3 and 6 pts were eventually assigned to the 1,200 and 1,800 mg, respectively. According to the FAB classification, 6, 2, and 1 pts were categorized to RAEB, RAEB-1, and CMML, respectively. There were 3 pts in each Int-2 and RAEB-1 groups, 2 and 1 pts in each risk group in the 1,200 and 1,800 mg arms, respectively. The median numbers of delivered cycles in the 1,200 and 1,800 mg arms were 4 (2 to 4) and 2 (1 to 8), respectively. DLT occurred in the 1,200 mg arm but in the 1,800 mg arm: 5 episodes of grade 3 non-hematological toxicities in 2 pts. One pt developed grade 3 C-reactive protein elevation, grade 4 granulocytopenia, grade 4 thrombocytopenia, and grade 3/4 neutropenia, as well as 1 case each of grade 3/4 thrombocytopenia, and grade 3/4 neutropenia, respectively. There were 3 pts each in the IPSS Int-1, Int-2, and high-risk groups, with 1 and 2 pts in each risk group in the 1,200 and 1,800 mg arms, respectively. The OS in patients aged >65 was 11 months, and 10 months in the group of anthracycline-cytarabine combinations, 3,81 months in chemotherapy plus allogenic transplantation, and 0.5 months in supportive measures group (Figure 1).

Summary/Conclusions: This Phase I study showed that intravenous rigosertib (1,800 mg daily) for consecutive 72 h was well tolerated, indicating that this is the RD for Japanese pts with MDS similar to a Phase III study in the U.S. Based on these clinical outcomes, Japanese pts with MDS are participating in a global randomized Phase III study to compare rigosertib with physicians’ choice of treatment.

PB1920

IRON CHELATION THERAPY IMPROVES HEMATOLOGICAL RESPONSE IN HIGH-RISK MYELODYSPLASTIC PATIENTS TREATED WITH AZACITIDINE
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Background: The goals of treating older patients with Myelodysplastic Syndrome (MDS) are different than for younger patients. Few elderly patients are able to pursue an allogeneic stem cell transplant. Azacitidine (AZA) improves the hematological remission, hematological improvement, and cytogenetic response were not obtained in the two arms. The Cmax values in the 1,200 and 1,800 mg arms were 5.99±1.50 and 10.00±2.81 μg/mL, respectively. The AUC0-∞ values were 314.6±142.7 and 324.6±83.9 μg·h/mL, respectively. Summary/Conclusions: The effects of rigosertib were performed in a routine clinical setting following Consensus Guidelines on Iron Chelation Therapy.

Aims: We report our experience on using the azacitidine in patients with high-risk MDS, evaluating the efficacy and safety. Concomitant treatment with deferasirox was performed in a routine clinical setting following Consensus Guidelines on Iron Chelation Therapy.

Methods: In our Institution from October 2009 to January 2017 we have...
treated 32 elderly patients (19 male and 13 female, median age 76 years, r. 71-88) affected by HIGH-RISK MDS (IPSS INT-2/HIGH). Patients received subcutaneous azacitidine at 75mg/m(2) daily for 7 days every 4 weeks. All patients completed at least 6 cycles of therapy, 12/30 (40%) patients underwent more than 8 cycles of therapy. 18/30 patients underwent as well iron chelation therapy with deferasirox receiving a starting dosage of 10 mg/kg/day, subsequently titrated according to serum ferritin (SF) measured monthly.

Results: Complete response (CR), partial response (PR), and hematologic improvement (HI) were observed in 2 (7%), 5 (17%), and 12 (40%) patients, respectively. The median number of cycles to clinical response was 4 (range 4-8). The 2-year rate of treatment-related mortality of acute myeloid leukemia-free survival was 48%. Five serious adverse events occurred in five patients with one fatal outcome. 16 out of 18 patients who showed any hematologic response (CR+PR+HI) meeting International Working Group 2006 criteria had also performed deferasirox therapy. No increased toxicity was noted when deferasirox was used concomitantly with azacitidine.

Summary/Conclusions: Our results confirm the effectiveness of the therapy with azacitidine in HIGH-RISK MDS elderly patients with acceptable toxicity profile. Peripheral cytopenias were the most commonly occurring adverse event, with gastrointestinal adverse events and injection-site reactions among the most commonly occurring non-haematological adverse events. In conclusion, azacitidine is an important agent for use in the treatment of elderly patients with MDS. Furthermore, concurrent use of deferasirox in patients with iron overload seems to significantly improve the hematologic response by reducing transfusion requirement.

PB1921
EXPLORING THE RISK OF RED CELL ALLOIMMUNIZATION IN MYELODYSPLASTIC SYNDROMES. TO WHAT EXTEND COULD CYTOTOGENETIC ANALYSIS AT DIAGNOSIS PREDICT THIS RISK? 1.T. Koutsavlis1,3, J. Falconer2, J. Fleming2, H. Riddle1
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Background: Red cell alloimmunization poses a huge burden for the blood transfusion services as it may be associated with crossmatching difficulties, haemolytic transfusion reactions and potentially severe clinical consequences for the transfused patient. Collectively, alloimmunization appears to be higher in patients with myelodysplasia (MDS) and chronic myelomonocytic leukaemia (CMML) with a rate somewhat around 15%. Identification of patients at risk of developing alloantibodies would be of clinical significance as antigen negative red cells could be crossmatched in advance for use in clinical practice. A large, comprehensive, cohort study of 228 cases was performed to explore whether the risk was increased in patients with poor or very poor cytogenetics as per IPSS-R. Descriptive statistics showed: very good/ good risk cytogenetics 69.7%, intermediate 12.7% and poor/ very poor 17.5%. Logistic regression analysis revealed no association between cytogenetic groups and risk of alloimmunization (p=0.89, p=0.96 and p=0.84 respectively).

Aims: To this end, we focused on exploring the cytogenetic profile from patients with MDS and CMML along with demographic characteristics as risk factors for alloimmunization.

Methods: A retrospective analysis was performed in 360 transfused patients with MDS (74.4%) and CMML (25.6%) registered in our local database between 1980 and 2016. Prognostic variables (age, sex, disease subtype) were assessed using a multivariate prediction model in SPSS statistical software. Cytogenetics at diagnosis were available in 226 of the above patients and uni- and variate analysis was performed separately.

Results: The mean age at diagnosis was 73 years (range 20-95) with 58.3% male patients. Overall, 45 patients (12.5%) formed 76 antibodies [88 alloantibodies, 8 autoantibodies] with 42% of them developing more than 1 antibody. 5 additional patients developed autoantibodies without alloantibodies. Alloantibody specificities were as follows: E (22 cases), C (8), K (7), Cw/Jka/Kpa (5 cases each), Lua (4), e/Fya (3 cases each), M (2), D/Chido/Bga (1 case each). Collectively, alloantibodies against the Rh and Kell systems encountered in 69% of this cohort. 6 out of 8 patients with anti-C had also developed a sec- ond antibody, whereas the model, notably the scoring involved, reached statistical significant level as predictors for immunization: age (p=0.59), sex (p=0.07), MDS WHO subtype (p=0.1). 228 patients had known cytogenetics at diagnosis. Normal profile (46, XY or 46, XX) was encountered in 58.8%. Similarly, univariate analysis of this cohort (normal versus abnormal cytogenetics) showed no significant difference (p=0.64). Further sub-group analysis was performed to explore whether the risk was increased in patients with poor or very poor cytogenetics as per IPSS-R.

Summary/Conclusions: The rate of alloimmunization in our cohort of patients was 12.5%, slightly lower compared to published studies. The most common alloantibody found was anti-E. Prognostic variables included in analysis (age, sex, cytogenetics, presence of iron overload) did not significantly impact the risk of alloimmunization and further studies are needed to investigate other possible risk factors. Prophylactic Rh and Kell antigen matched cells, when possible, would be a reasonable strategy until further knowledge is acquired.

PB1922
PROGNOSTIC MARKERS THAT PREDICT THE OUTCOME OF REDUCED INTENSITY CONDITIONING TRANSPLANT IN ADULT PATIENTS WITH MYELODYSPLASTIC SYNDROMES: A SINGLE CENTER EXPERIENCE S. Elaslawih1, S. Shama2, H. Kamei3, M. Sama4, E. Azmy1
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Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of hematologic diseases, characterized by a clonal abnormality of hematopoietic stem cells. The incidence of MDS is age-dependent. The treatment approach is to categorize patients into lower or higher risk MDS and to select a suitable treatment accordingly. HCT offers potentially curative therapy for patients with MDS. The most intensified conditioning (RIC) regimen was used to reduce the toxicities associated with transplant procedure. The main concept of RIC relay upon adoptive immunotherapy especially in the low risk patients allowing the graft versus leukemia to occur.

Aims: This study aimed to investigate the outcome of allogeneic peripheral blood stem cell transplantation among the intensity conditioning patients (RIC) in adult patients with MDS, the effect of different prognostic factors on outcome and the effect of chronic GVHD according to IPSS risk.

Methods: A retrospectively study analyzed the fifty-one patients with MDS who underwent transplantation at the BMT unit at Nasser Institute during a period of 6 years, presence of GVHD significantly improved OS especially in high-risk patients, blood stem cell. Outcomes analyzed the incidence of acute and chronic GVHD, disease free survival (DFS) & overall survival (OS).

Results: They were 31 males (60.8%) and 20 females (39.2%). Their ages ranged from 17 to 60 years, with mean age±SD of 42±10.1 years. The frequency of abnormal cytogenetics (del(5q), -Y in 6 patients, del(7q), del(11q), del(20q)) was significantly higher in patients with MDS as compared to healthy controls. The most common cytogenetic abnormalities were del(5q) in 26 patients (51%), del(7q) in 21 (41%), del(11q) in 13 patients (25.5%), del(20q) in 2 patients (4%). According to IPSS, 10 patients (21.6%) were low risk, 28 patients (54.9%) were intermediate-I risk group, and 9 patients (17.5%) were intermediate-II & 3 patients (5%) were high risk group. The incidence of acute and chronic GVHD were 51.1% and 28.6% respectively. The 5-year estimate for overall survival of the whole group was 21.8%. In univariate analysis, covariates associated with a better OS were recipient age <40 years (p=0.02) and the presence of cGVHD (p=0.002). On multivariate analysis the presence of cGVHD is significant predictor of survival (p=0.04). Also cGVHD significantly improve the OS for low and high risk MDS group (p= 0.02 and 0.03 respectively). While presence of acute GVHD, IPSS & interval between diagnosis and transplant weren’t significantly affect OS (p>0.05). The 5-year estimate for DFS of the whole group was 28.6%. On multivariate analysis the presence of cGVHD significantly reduce relapse (p=0.029).

Summary/Conclusions: The presence of cGVHD significantly improved OS and reduced the risk of relapse in patients with MDS. We also found that the presence of cGVHD significantly improved OS especially in high-risk patients group, which suggests that the GVCL effect may be beneficial in high-risk patients who do not receive intensive preparative regimens.
Background: Myelodysplastic syndromes (MDS) are included into a heterogeneous group of clonal blood diseases characterized by peripheral cytopenias, dysplastic features of hematopoietic precursors, progressive deterioration and a high risk of transformation into leukemia. MDS occur in several subtypes that differ in frequency of appearance, the duration of the course and the probability of transformation into acute leukemia. The choice of therapy for a particular patient is determined by the morphological variant of the disease, the prognostic group, age and comorbidity. In hypoplastic cases of MDS are often used immunosuppressive therapy.

Aims: Analysis of the effectiveness of immunosuppressive therapy in patients with primary MDS

Methods: The research included 19 patients with primary MDS from 22 to 58 years (median age 46 years, 11 male, 8 female). The diagnosis was made according to the criteria of the WHO classification of the WHO classification in 2008. The materials were taken only after signing by patients informed consent form to participate in the research. The calculations are performed in the R version 3.1.3 statistical package.

Results: There were patients with defined MDS subtypes: RA in 52.6%, RCMD in 31.8, RAEB in 15.8% Hypoplastic forms of MDS were diagnosed in 63.2% patients. The increased number of lymphocytes in the bone marrow of patients were 52.6%, accumulation of lymphocytes in the bone marrow biopsy – in 36.8%. Cytogenetic abnormalities were found in 21% of patients (in 5.3% complex and in 15.7% isolated). All patients used immunosuppressive therapy as a first-line treatment: Antithymocyte globulin and Cyclosporine A (CsA) in 15.8%, monotherapy with CsA in 84.2%. CsA therapy started at a dose of 5 mg/kg per day. Dose correction performed depending on the concentration of CsA in the serum and toxicity. Median treatment was 143 days (36...1253 days). The response rate to CsA treatment was considered a complete remission (normalization of blood and bone marrow), partial remission (improvement of blood counts for more than 50% and no dependence on transfusions of blood components) or improvement (reduction in transfusion requirements by 50% or more). Complete remission was achieved in 10.5% of patients (only variant RA). Partial remission was obtained in 31.8% (variants RA and RARS and RAEB). There was no response to treatment in 21,1% of patients (variants RCMD and RAEB). Positive effect on immunosuppressive therapy significantly more likely achieved in patients with hypoplastic forms MDS (57,9%) and the presence of clusters of lymphocytes in the bone marrow biopsy (36.8%). Dependence of treatment efficiency and cytogenetic-phenotypic abnormalities not detected.

Summary/Conclusions: The effectiveness of immunosuppressive therapy in MDS associated with a variant of the disease, bone marrow cellularity and the bone marrow lymphoid infiltration. The greatest effect of the immunosuppressive therapy can be expected in patients with hypoplastic MDS and accumulation of lymphocytes in the bone marrow biopsy.

PB1926

VITAMIN D IS ASSOCIATED WITH SEVERITY OF DISEASE AS EXPRESSED BY SUBDIAGNOSIS AND IPSS-R IN PATIENTS WITH MYELODYSPlastic SYNDROMES AND RELATED DISEASES

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Background: Recent findings indicate that vitamin D (VD) might impact hypomethylating therapy of myelodysplastic syndromes (MDS). Epigenetic activity of VD is mainly mediated through interaction with its nuclear receptor (VDR). Activated VDR binds to specific genomic sequences (VD response elements) which influence gene transcription by histone modification, mainly acetylation but also demethylation. Among genes affected by VDR/VDR is BGLAP expression but also demethylation. Among genes affected by VDR/VDR is BGLAP expression but also demethylation. Among genes affected by VDR/VDR is BGLAP expression but also demethylation. Among genes affected by VDR/VDR is BGLAP expression but also demethylation.

Aims: We initiated an exploratory study, collecting patients' data on serum VD, and osteocalcin (OCN) levels in 59 selected patients with MDS, MDS/myelo-proliferative neoplasm (MPN) and secondary acute myeloid leukemia (sAML).

Methods: Serum VD levels were assessed by measuring 25-hydroxyvitamin D (25(OH)D), the biochemical indicator of VD status. Analysis was done by clinical chemistry immuneassay. Intact OCN is unstable due to cysteine cleavage between amino acids 43 and 44. The N-MID-fragment, resulting from cleavage, is considerably more stable. Measurement of both intact OCN and stable N-MID-fragment was effectuated by electrochemiluminescence immunoassay.

Results: We found median serum 25(OH)D levels (normal range 30-100 ng/ml) of 16 ng/ml (RA, RARS, n=35), 23 ng/ml (RAEB-1/2, sAML, n=16), and 20 ng/ml (MDS/MPN, n=8) (p=0.273).When classified by IPSS-R, median serum 25(OH)D levels were 18 ng/ml in ("very low") (n=20), 16.5 ng/ml in
“intermediate” (n=14), and 29.5 ng/ml in “(very) high” (n=6) with p=0.102. Regarding cytogenetic risk classification median serum 25(OH)D levels were 18 ng/ml in “(very) good” (n=48), 19 ng/ml in “(very) low” (n=20), 16.5 ng/ml in “intermediate” (n=14), and 29.5 ng/ml in “(very) high” (n=6) with p=0.102. Regarding cytogenetic risk classification median serum 25(OH)D levels were 18 ng/ml in “(very) good” (n=48), 19 ng/ml in “(very) low” (n=20), 16.5 ng/ml in “intermediate” (n=14), and 29.5 ng/ml in “(very) high” (n=6) with p=0.102. Regarding cytogenetic risk classification median serum 25(OH)D levels were 18 ng/ml in “(very) good” (n=48), 19 ng/ml in “(very) low” (n=20), 16.5 ng/ml in “intermediate” (n=14), and 29.5 ng/ml in “(very) high” (n=6) with p=0.102. Regarding cytogenetic risk classification median serum 25(OH)D levels were 18 ng/ml in “(very) good” (n=48), 19 ng/ml in “(very) low” (n=20), 16.5 ng/ml in “intermediate” (n=14), and 29.5 ng/ml in “(very) high” (n=6) with p=0.102. Regarding cytogenetic risk classification median serum 25(OH)D levels were 18 ng/ml in “(very) good” (n=48), 19 ng/ml in “(very) low” (n=20), 16.5 ng/ml in “intermediate” (n=14), and 29.5 ng/ml in “(very) high” (n=6) with p=0.102.
patients. All patients required erythropoiesis stimulating agents and 9 patients received treatment with azacytidine (AZA), including all the Int-2 patients and 3 lower risk patients who progressed to a higher risk MDS. Estimated cumulative survival at 46 months was 67% with a median OS not reached and median follow-up time of 34 months. Patients receiving AZA revealed a trend towards survival benefit (mean survival 54.2 vs 50 months), independent of IPSS and R-IPSS. The difference was not significant. Multivariate analysis revealed that 75.6% of patients had at least one gene mutation and it was most frequently related to DNA methylation genes (n=14), particularly in PET2 (n=7 patients) and DNMT3A (n=6 patients, 7 different mutations). We found a statistically significant difference between mutations in these genes and lower absolute neutrophil count (n=0.047, G=21, PL=0.4). The most frequently mutated genes were related to signal transduction pathways (n=11; JAK1, JAK2, NRAS, CBL, GATA2, SH2B3, CSFR). Patients with these mutations had significantly lower serum EPO levels (p<0.001; median 32.35 vs 42.70 UI/L). Furthermore, patients with such mutations demonstrated a clear discrepancy from analysis, with a median OS of 19 months for patients not reached with mutations (p<0.001), being these results independent of the IPSS and R-IPSS risk groups. We were also able to identify a trend towards worst survival in patients with previously described high risk mutations (IPSS, EZH2, ASXL1, RUNX1 and ETV6 genes).

Summary: We conclude that the most frequently detected mutations were related to DNA methylation genes, as described in the literature, which was independent of the IPSS risk group, being observed in both low-risk and high-risk patients. These results raise the question whether hypomethylating agents may also be of benefit for lower-risk patients. We suggest that all patients with signal transduction pathways which was related to a clear survival disadvantage across all risk groups of the IPSS and R-IPSS. This unveils the question we may be facing a shift towards the molecular level in MDS risk stratification and if therapies targeted to such molecules may improve the outcome of these patients.

**PB1930**

**CLINICAL FEATURES, CYTOGENETIC STUDY AND OUTCOME OF ADULT MEYLODYSPLASTIC SYNDROMES: REVIEW OF 101 CASES, A SINGLE CENTER EXPERIENCE IN ALGERIA.**

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**Background:** Myelodysplastic syndromes (MDS) are heterogeneous disorders defined as clonal diseases involving hematopoietic stem cells and even characterized by cytopenias, with a high risk of leukemic transformation. Morphological analysis of peripheral blood (PB) and marrow aspirates or bone marrow biopsies is the first step that ensures a diagnosis of MDS. Cyto genetic studies are important means of defining different prognostic groups and even of showing how patients are eligible for this or that treatment. We conducted the first study including conventional karyotyping and fluorescence in situ hybridization (FISH) for MDS in our country.

**Aims:** Our study was aimed to evaluate outcome of MDS regarding IPSS and IPSS-R classification in an emerging country.

**Methods:** Between January 2012 to December 2016, 101 patients with MDS were consecutively diagnosed. Frequent genetic abnormalities in MDS were scoring R-IPSS, with high frequency of mutations ≥7 in signal transduction pathways which were related to a clear survival disadvantage across all risk groups of the IPSS and R-IPSS. This unveils the question we may be facing a shift towards the molecular level in MDS risk stratification and if therapies targeted to such molecules may improve the outcome of these patients.

**Results:** Among these 101 pts, 58 were male with a sex ratio=1, 35; range in age is from 18 years to 94 years with a median of 61, 6 years. Median hemoglobin value was 80 g/L (29-150), more than 60% of patients had severe anemia with anemia defined as anemia of chronic disease (n=7), thalassemia (n=15), iron deficiency (n=24), myelodysplastic syndrome (n=6). Blood cell count was abnormal in 95% of cases fludarabine and cytarabine containing regimens are also used. In the last ten years, in the context of a clinical trial, a series of patients have received a less intensive, hypomethylating therapy (repeated courses of 5-azacytidine 75 mg/m2 subcutaneously for 7 days), as bridge to transplant. Conditioning regimens used in MDS patients is busulfan based in younger patients (BuFlu, BU-Cy); in the elderly or less fit patients a RIC regimen (thiopepa 5 mg/kg e.v., fludarabine mg/m2 x 3 and L-PAM 100 mg/m2) is administered.

**Results:** In the last ten years we performed 14 HCT (between June 2008 and set from 2016) in patients with MDS with excess blasts. Median patient’s age was 63,5 years (range: 49-69), female/male ratio was 9/5. According to IPSS, 12 out of 14 patients were high/Int-2 (2 Int-1), 11/14 had >10% blast cells (EB-2). According to our centre protocol, we treated 11 patients with EB-2 and 1 patient with EB-1 (with hypercellular bone marrow) with a debulking therapy. This was I.C. in 8 patients and 5-AZA in 6 patients. Two patients with EB-1 did not receive any therapy pre-transplant. However, both of them are not evaluable, due to early mortality. Transplant conditioning was RIC in 11/14 patients, myeloablative in 3 cases. The donor was a sibling in 9/14, MUD in 5/14. Four out of six patients treated with I.C. achieved a pre-transplant CR (87%), compared to one out of six in the 5-Aza cohort (17%). Four patients who experienced a relapse post HCT, after a median of 8,5 months (4-11). With a median follow up of 21 months (6-68), post transplant RR was 4/12 (33,3%) and was not influenced by debulking therapy (I.C. vs 5-Aza, p=0,54), nor by pre-transplant disease stage (CR vs noCR, p=0,22). In fact, 3 out of 6 patients treated with I.C. progressed, but only 1 out of 6 treated with 5-Aza relapsed after transplant. Three out of four patients who subsequently relapsed had received RIC transplant; type of transplant was not associated with relapse (P=1,0). The only variable that showed a trend for reduced RR was MUD transplant (p=0,08).

**Summary/Conclusions:** Extreme caution must be used in considering our data, given the very small patients number. In our cohort, pre-transplant intensive debulking chemotherapy, although obtained an high rate of CR, showed no effect in preventing relapse. Larger studies are necessary to assess the real utility of I.C. in this subset of frail patients.

**PB1932**

**IRON CHELATION THERAPY IN MEYLODYSPLASTIC SYNDROMES AND IN OTHER TRANSFUSION-DEPENDENT CHRONIC ANEMIAS: RETROSPECTIVE STUDY OF 69 PATIENTS FROM A SINGLE INSTITUTION.**

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**Background:** Although several recent guidelines recommend iron chelation therapy (ICT) for iron overload in transfusion-dependent patients (pts) with lower-risk myelodysplastic syndromes (MDS), several barriers may limit the initiation or the continuance of ICT: older age, comorbidities, poor tolerance and compliance.

**Summary/Conclusions:** Our results are in agreement with those previously published regarding demographic factors, distribution of pre-treatment cytogenetic abnormalities and prediction of survival. Myelodysplasias are among the most difficult hematological diseases to treat. Treatment of low risk and high risk myelodysplasia are completely different, the last group carrying a great risk of leukemic transformation. For all these reasons, application of the new tools to R-IPSS, but not Strobe, in the next future may be helpful.

**Methods:** In our Institute, we treat all patients with a blast cell count of 10% or higher with a pre-debulking therapy pre-transplant. This is usually an AML-like, cytarabine and anthracycline based, intensive chemotherapy (I.C.). In selected cases fludarabine and cytarabine containing regimens are also used. In the last ten years, in the context of a clinical trial, a series of patients have received a less intensive, hypomethylating therapy (repeated courses of 5-azacytidine 75 mg/m2 subcutaneously for 7 days), as bridge to transplant. Conditioning regimens used in MDS patients is busulfan based in younger patients (BuFlu, BU-Cy); in the elderly or less fit patients a RIC regimen (thiopepa 5 mg/kg e.v., fludarabine mg/m2 x 3 and L-PAM 100 mg/m2) is administered.
Aims: Therefore, with the aim of assessing the safety and efficacy of ICT in the daily clinical practice, we retrospectively analyzed our single-center experience on ICT in MDS and other chronic anemias.

Methods: From October 1997, in our Institution, 69 pts (48 males), median age: 74 (23-96) yrs, with transfusion-dependent anemia, received ICT, because of a diagnosis of iron overload, i.e. both a transfusion history of at least 20 units of RBC and a serum ferritin (SF) higher than 1000 ng/ml.

Results: 40 pts (58%) were affected by lower-risk MDS (IPSS risk: low or intermediate-1), while 13 pts (18.8%) showed a higher-risk MDS (IPSS risk: high or intermediate-2) but were considered for ICT because of responsiveness to hypomethylating therapy and/or eligibility for allogeneic SCT. 16 pts (23.2%) were affected by other diseases (chronic myelomonocytic leukemia: 2 pts; idiopathic myelofibrosis: 3 pts; aplastic anemia: 9 pts; pure red cell aplasia (PRCA): 2 pts). 45 pts (65.2%) received deferasirox (DFX) as first-line treatment, 12 pts (17.4%) received DFX after a previous treatment with deferoxamine (DFO), while 9 pts (13%) received DFO and 3 pts (4.3%) received DFO after DFX because of contraindications to DFX or toxicity. Median time from diagnosis to the start of ICT: 18 months. Median number of RBC transfusions before the start of ICT: 3.75. Median SF level pre-ICT: 1964 ng/ml; median SF after ICT (last value): 1858 ng/ml; median duration of ICT: 12 (range 1-230) months. 36 pts (52.2%) continued ICT for a period ≥24 months, and 25 pts (36.2%) for a period ≥24 months. 27 pts (39.1%) showed a decrease of SF ≥500 ng/ml, 11 pts (15.9%) showed a drop of SF <500, 13 pts (18.8%) showed an increase of SF <500, in spite of ICT, and 18 pts (26.1%) showed an increase of SF ≥500. 12 pts (17.4%) achieved a SF value <1000, and 48 pts (69.6%) a SF value <2500. Adverse events possibly related to DFX were observed in 30 pts (43.5%): renal (increase of serum creatinine): 14 pts (20.3%) (grade >2: 1 pt: 1.4%); gastrointestinal : 14 pts (20.3%) (grade >2: 1 pt: 1.4%); cutaneous: 2 pts (2.9%) (grade >2: no pts). Permanent discontinuation of ICT: 40 pts (58%), because of toxicity (16 pts: 23.2%), worsening of clinical condition (6 pts: 8.7%), discontinuation of transfusions (9 pts: 13%), allogeneic transplantation (9 pts: 13%), 5 pts (7.2%) (4 MDS and 1 PRCA) (with DFX: 4 pts; with DFO: 1 pt) showed an erythroid response following ICT, after 2, 4, 7, 32 and 112 months, respectively, and one of them (with PRCA) achieved complete remission. 35 pts (50.7%) died, because of infection (9 pts), AML (4 pts), cachexia (4 pts), other acute/chronic diseases (6 pts), hemorrhage (2 pts), heart failure (2 pts), stroke (2 pts) and other causes (9 pts). 10 pts (14.5%) are still receiving ICT. With a median follow-up of 34 (2-230) months, median overall survival (OS) was 64 months for all pts, 51 months for MDS pts, 87 months for lower-risk MDS (IPSS risk: low or intermediate-1), 64 months for intermediate-2 and high.

Summary/Conclusions: In conclusion, in our experience ICT appears feasible and effective, in terms of reduction of SF and OS, even in a population of elderly pts, if carefully selected.

Myeloma and other monoclonal gammopathies - Biology

PB1933

VCAM-1 AS A NOVEL DRUG THERAPY TARGET OF BONE MARROW MESENCHYMAL STEM CELLS IN MULTIPLE MYELOMA

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Background: Multiple myeloma is characterized by the clonal proliferation of malignant plasma cells in the bone marrow microenvironment. The pathogenesis of MM is, in part, related to pathological interactions between myeloma cells and the mesenchymal stem cells (MSC). The interactions between myeloma cells and bone marrow cells are established through surface receptors (e.g. integrins, cell adhesion molecules, etc.), which determine tumor growth, survival, migration and drug resistance. Mesenchymal stromal cells modulate the pattern of myeloma markers on the cellular surface in vitro towards a less differentiated phenotype. However, the exact mechanism by which mesenchymal stromal cells carry out their functions is not yet fully understood.

Aims: To evaluate the effect of MSCs from healthy donors and myeloma patients over malignant plasma cells and the cellular changes produced for the interaction each other.

Methods: Interactions between both cell types were studied through different co-cultures studies. We evaluate differences between culturing primary MSC and MM cell line RPMI 8226. Pathological MSCs were extracted from the bone marrow of newly diagnosed MM patients. On the other hand, purified healthy MSCs will be isolated from donor patients. Pathological or healthy MSCs were cultured and co-cultured 24h after seeding with MM plasma cells RPMI 8226 for duplicates at 24, 48 and 72h. The phenotypical and molecular effect of the interaction of both cell types was characterized by viability through trypan blue, cell apoptosis percentage (7AAD) and variations on expression of cell surface markers (MSCs: CD90, CD105, CD166, CD54, CD44, CD11a, CD49d, CD62E, CD80, CD86, VCAM-1) using flow cytometry, and statistically analyzed with GraphPad.

Results: We observed a decrease of apoptosis of MM plasma cells when are in co-culture with pathological MSCs at short-term (24h, 7AAD positive cells MM alone: 4.8%, MM in co-culture: 0.4%) and mid-term (72h, 7AAD positive cells MM alone: 16.4%, MM in co-culture: 10.7%) compared with MM plasma cells alone. However MM plasma cells do not decreases the level of apoptosis at mid-term with healthy MSCs in co-cultures (72h, 7AAD positive cells MM alone: 16.4%, MM in co-culture: 18.0%). The molecular analysis showed a correlation between MSC lack of protection over MM plasma cells and the decrease in the levels of expression of VCAM-1 (CD106).

Summary/Conclusions: As reported in literature CD106 expression increase when MSCs are co-cultured with plasma cells. Adhesion of tumor cells to BMSC activates many pathways resulting in upregulation of cell cycle and anti-apoptotic proteins. MM pathophysiologhy is supported by a strong interaction between CD106/CD49d. Changes in VCAM-1 and VLA-4 expression have been demonstrated in cell lines assays, and were corroborated with primary cells in the context of MSCs protection over MM plasma cell. Thus, MM pathological MSCs did not change VCAM-1 levels and MM plasma cell protection be held. However, healthy MSCs have the capacity to modulate the VCAM-1 in mid-term to avoid the protection effect. Therefore, these results suggest MSCs VCAM-1 as potential drug therapy target in MM disease.

PB1934

RALA AND RALB MEDIATE CELL SURVIVAL INDEPENDENTLY OF ONCOGENIC RAS AND PROVIDE POTENTIAL THERAPEUTIC TARGETS IN MULTIPLE MYELOMA

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Background: Genetic mutations and the bone marrow microenvironment contribute to disease progression, aggressive phenotype, and shorter survival in multiple myeloma (MM). Oncogenic RAS is one of the most common mutations in MM. Pathway activation through oncogenic RAS is associated with promotion of disease progression and shorter survival. Cell survival and proliferation in MM are mainly mediated via classical signaling pathways such as MEK/ERK and PI3K/Akt. Since there is a lack of specific RAS-inhibitors for clinical use, it is important to identify and analyze associated pathways, which may provide useful alternative targets for MM therapy. The small GTPase Ra1 has previously
been implicated in putative downstream signaling of RAS, and may therefore promote tumor proliferation and survival of MM cells.

**Aims:** We used shRNA-mediated knockdown of RalA and RalB isoforms to appraise their role as potential therapeutic targets and to analyze their connection to important signaling pathways, which regulate MM cell survival and proliferation. Because oncogenic RAS is a potential activator of the Ral pathway, we invesigated the relationship between oncogenic RAS and Ral signaling.

**Methods:** Immunohistochemical stainings of bone marrow trephines of MM patients and Western analysis of primary MM cells and MM cell lines were performed to evaluate Ral protein expression. Transient or stable knockdown of RalA or RalB was achieved by electroporation of MM cell lines and the effect on cell proliferation was measured with flow cytometry using annexin V/propidium iodide staining. Ral pulldown assays were applied to test potential dependence of Ral activation on oncogenic RAS. Furthermore, RNA sequencing was performed to compare RAS and Ral gene expression signatures after respective knockdowns.

Results: Both Ral variants were expressed in primary MM cells and MM cell lines, with RalA showing the most prominent and consistent protein expression levels. ShRNA-mediated knockdown of RalA strongly induced apoptosis in two thirds of the tested cell lines, whereas RalB depletion did impair MM cell survival in less than half of the cell lines. Western analysis revealed no alteration of classical MAPK pathway activation after Ral knockdown. Ral activity appears to be independent of oncogenic Kras or NRas. In addition, RNA sequencing revealed differing gene expression signatures for RAS and Ral.

**Summary/Conclusions:** Ral and its effector network constitute potential therapeutic targets in MM, which are activated independently of oncogenic K- or NRAS. Therefore, investigation of the functional network of Ral may be important to identify useful clinical targets.

**PB1935**

**CXCR4 MUTATIONS FOUND BY USING DEEP SEQUENCING WITHOUT SORTING B CELLS, AND PROGNOSTIC IMPLICATION IN WALDENSTROM MACROGLOBULINEMIA**

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**Background:** Waldenstrom macroglobulinemia (WM) is a lymphoplasmacytoid lymphoma with IgM monoclonal gammapathy. Most of WM harbor MYD88 L265P and one third of WM with MYD88 present CXCR4 mutations. Currently, frequency of CXCR4 mutations and its clinical implication is not reported in Asian patients with WM.

**Aims:** We investigated the profiles of CXCR4 and MYD88 mutation in correlation with prognostic implication. To detect minor cell population with CXCR4 mutation, we adopted a ultra-deep sequencing strategy for CXCR4, which can detect mutation variants 1% of the cell population.

**Methods:** Allele-specific PCR for MYD88 was performed on 37 patients with WM, along with 161 patients with B-cell neoplasms [diffuse large B-cell lymphoma (DLBCL), B-cell acute lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia (CLL)]. Deep-sequencing for WM, along with 161 patients with B-cell neoplasms [diffuse large B-cell lymphoma (DLBCL), B-cell acute lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia (CLL)], and 133 healthy persons were performed. Deep-sequencing for CXCR4 mutation was performed on 37 patients with WM. Clinico-pathologic features were compared among 3 groups according to MYD88 and CXCR4 mutation status (Group 1, MYD88WT and CXCR4WT; Group 2, MYD88L265P and CXCR4WT; Group 3, MYD88L265P and CXCR4Mutation; statistical comparison, Fischer exact test, one-way ANOVA).

**Results:** MYD88 L265P mutation was detected in 81.3% (26/32) patients with WM, 10.8% (9/83) in patients with DLBCL, 9.5% (6/63) in patients with CLL, 0% (0/15) in patients in B-ALL, and 0% in 200 healthy persons. Among the 31 WM patients, 6 patients have CXCR4 mutation (19.4%) in the c-terminal domain (Figure 1); 1 frameshift mutation and 5 nonsense mutations. Two CXCR4 frameshift mutations are novel. Both coding (36-37) and non-coding (141-142) sites were 5' flanking regions. All of them had MYD88 L265P mutation. FISH revealed 6q21 deletion in 14 patients (43.8%), and IGH rearrangement in 9 patients (28.1%). There was no correlation among cytogenetic aberrations and genetic mutation (MYD88 and CXCR4). IgM levels of group 2 (MYD88L265P and CXCR4WT) were significantly higher than that of group 1 (MYD88WT and CXCR4WT) (P=0.024). Meanwhile, IgG level was significantly lower in group 1, compared to group 2. Other clinical characteristics such as age, Hb, platelet, adenopathy, hyperviscosity showed no significant difference among 3 groups. Group 1 showed adverse survival and 1 year survival rate of group 1 (68.7%) was lower than that of group 2 (84.7%), though it was not statistically significant (P=0.410). There were no death events in group 3 (MYD88L265P and CXCR4Mutation) during the research period.

**Summary/Conclusions:** The frequency of CXCR4 mutation in Korean WM was similar to those of Caucasian. We suggest that ultra-deep sequencing using deep-sequencing can detect the CXCR4 mutation, which is a strong enhancer of MYD88 protooncogene (located at 16q23) under strong enhancer of IgH gene (14q32). Although the IgH/MHF positive cases comprise just 2-4% of MM patients, the evaluation of this aberration is an integral part of the cytogenetic risk stratification according the RISS. The tumorigenesis of IgH/MHF MM is considered to be a reliability of MYD88. We failed to detect CXCR4 mutation in WM as having at least one of the following aberrations: deletion of 17p13 (TP53 gene), translocation t(14;16)(p13;q32) and translocation t(14;16)(q32;q23) determined by FISH. However, the unequivocal poor prognostic value of t(14;16)(q32;q23) was not confirmed in several MM series thus further studies are needed.

**Aims:** The aim of our study was to assess the impact of t(14;16)(q32;q23) on event free (EFS) and overall survival (OS) in cohort of IgH/MHF positive MM patients in comparison with control group of 30 MM IgH/MHF negative cases.

**Methods:** During the years 2004 to 2016, we examined 870 bone marrow samples of MM patients on immunofluorescently labeled plasma cells (clg FISH). The basic FISH panel included 4 specific DNA probes (Abbott-Vysis, Kreatech and MetaSystems) detecting: the IgH gene rearrangement (1), deletion 13q14 (RB1 gene/monosomy 13 (2), gain of 1q21/deletion of 1p32 (3) and deletion of TP53 gene (4). Cases with rearranged IgH gene were gradually examined for 3 specific translocations- t(1;11)(q13;32), t(4;14)(p13;q32) and t(3;14)(q13;32). Kaplan-Maier analysis was performed to evaluate OS and EFS.

**Results:** Translocation t(14;16) was identified in 19 out of 870 patients (2.2%). Eighteen patients were examined at the time of diagnosis and one at the time of the progression of asymptomatic myeloma to symptomatic disease. Relapse and/or disease progression occurred in 15 patients. The median event-free survival (EFS) was 13 months in t(14;16) carriers (range 3 – 62 months) and 22.5 months in controls (range 3-71 months). Healthy Fourteen (14;16) positive patients died. The median overall survival (OS) was 25 months (range 10-204 months) in comparison with 52 months in control group (range 3-132 months). However, the difference in OS was not statistically significant (p=0.155). In 15 (14;16) positive patients (83.3%), two or more additional chromosomal changes were detected by FISH (monosomy/deletion of chromosome 13 being the most frequent). In four cases, (14;16) was detected together with another high risk chromosomal change - deletion of TP53 gene - and all these patients died within median of OS 12.5 months (range 10-16).

**Summary/Conclusions:** Beside its supposed negative clinical impact, the examination of t(14;16) is not always included in routine diagnostics of chromosomal changes and its prognostic significance should be proved in large series of MM patients. Our data substantiate the trend of worse clinical outcome (shorter OS) in t(14;16) positive group compared to IgH/MHF negative MM patients. The detailed analysis of other clinical parameters, type of therapy, combination with other chromosomal aberrations will be performed to prove its role as an independent prognostic factor.

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THE ROLE OF NEUROTROPHINS AND ANGIOGENIC CYTOKINES IN THE PATHOPHYSIOLOGY OF PERIPHERAL NEUROPATHY IN PATIENTS WITH MULTIPLE MYELOMA

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Background: The introduction of new treatment modalities has changed significantly the prognosis of multiple myeloma (MM) patients. The novel drugs and schemes of treatment of MM have contributed to substantial extend of the overall survival time of patients. However, the administration of some of the treatment regimens or bortezomib or dexamethasone is also associated with occurrence of a serious and common side-effect problem, which is the drug-induced peripheral neuropathy. The mechanism of the development of the peripheral neuropathy is poorly understood. Nevertheless, one of its potential cause, could be inadequate concentrations of crucial trophic factors, including neurotrophic and angiogenic factors, which are responsible for proliferation, differentiation, survival and death of neuronal and non-neuronal cells.

Aims: The aim of this study was to elucidate the potential relationship between concentration of neurotrophic and angiogenic factors and development of peripheral neuropathy in the natural clinical course of the disease and, especially, induced by treatment regimen: VMP (bortezomib, melphalan, prednisone) or VTD (bortezomib, thalidomide, dexamethasone) in patients with MM.

Methods: Peripheral blood samples were collected from patients classified into two groups: i) patients with multiple myeloma, without neuropathy and before therapy; and ii) patients with peripheral neuropathy 3 or 4th induced in the course of VMP or VTD therapy. The control group consisted healthy age-matched subjects. Assessment of concentrations of neurotrophins (BDNF, NSE) and angiogenic factor (PDGF) were performed using Luminex technology, which utilize microbeads coated with fluorescently labeled antibodies.

Results: Concentration of BDNF, PDGF and NSE were significant decreased in patients after treatment regimen involving VMP or VTD who have developed peripheral neuropathy grade 3 or 4, compared with patients with newly diagnosed MM without neuropathy, before therapy and control healthy group. Additionally, plasma levels of both neurotrophins and PDGF in patients before therapy were higher, then in control group. Obtained results may be caused by the changes in an activity of the transcription factor NF-kB during the treatment of MM, since reduction of NF-kB concentration is associated with decrease in the transcription of genes encoding BDNF, NSE and PDGF.

Summary/Conclusions: Alterations in the concentration of BDNF, PDGF and NSE suggest the cause and effect relationship between these factors and the development of neuropathy in patients with MM. Comprehensive elucidation of this phenomenon may contribute to the extension of the knowledge concerning the pathogenesis of neuropathy, and might well lead to reduction of the incidence of polyneuropathy in MM patients in the future.

PB1938

INFLUENCE OF XRCC5, XRCC4, NFKB2, AND BIRC5 GENES POLYMORPHISMS IN THE RISK AND PROGNOSIS OF MONOCLONAL GAMMOPATHIES

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Background: Monoclonal gammopathies (MG) are a group of disorders characterized by the proliferation of monoclonal plasma cells, which produce and secrete monoclonal immunoglobulin (M protein). Symptomatic multiple myeloma (MM) is defined by the clonal proliferation of plasma cells. MM is consistently preceded by a pre-neoplastic entity, called monoclonal gammopathy of undetermined significance (MGUS), with an intermediate phase of indolent multiple myeloma (MMI). This disease is a heterogeneous hematological neo-plasm characterized by the proliferation of clonal, long-lived plasma cells within the bone marrow (BM) secreting monomolecular proteins and by the presence of so-called CRAB criteria and/or biomarkers of malignancy (as clonal BM plasma cells > 10%, involved/uninvolved serum free light chain ratio >100, > 1 focal lesion in MRI studies). Genetic instability and several molecular abnormalities are hallmarks of MM cells. Alterations in DNA repair pathways, namely abnormal activity of non homologous end–joining (NHEJ) repair pathway, are involved in the disease onset and progression. Moreover, it has been observed that virtually all primary MM samples have constitutive nuclear factor-κB (NF-κB) pathway activity, having this pathway a well-established role in MM pathogenesis.

Aims: To explore the role of core genes involved in NHEJ repair pathway (XRCC5, XRCC4) and in NF-kB pathway (NFκB2, and BIRC5) in disease MG and MM.

Methods: In the present, a hospital-based case-control study, we analyzed eight polymorphism in four genes (XRCC5, XRCC4, NFKB2, and BIRC5), by genotyping 189 individuals (63 MG patients and 126 controls) using TaqMan qPCR. Results are expressed in terms of frequencies of allele, genotype, haplotype, and genotypic profiles, and their correlation with MG susceptibility. The strength of association between polymorphisms and disease risk was assessed by odds ratio (OR) with 95% confidence interval (CI(95%)) calculated by logistic regression. We also investigated the association of these SNPs with overall survival through Kaplan Meier curves. All statistical analyses had a significance level of 95%.

Results: In the patient group, 51% (32/63) of the individuals were females and 49% (31/63) were males; the mean age was 70.1±10.25 years old. Among the MM patients, 52% (65/126) of the individuals were females and 48% (61/126) were males, and the mean age was 69.9±10.06 years old. Most of the patients were diagnosed with multiple myeloma (84%), 53/63) and the remaining ones (16%, 10/63) were diagnosed with smoldering multiple myeloma. According to the ISS classification, 43% (27/63) of patients are in stage III. The data analysis revealed two associations of the studied gene polymorphisms with MG. First, the analysis by gender stratification suggested a decreased predisposition to MG in male carriers of NFκB2 rs12769316 GA and AA genotypes (OR 0.346, 95CI: 0.124–0.965, p=0.043). Second, we observed that patients with BIRC5 rs9904341 CC genotype had a highly significant lower overall survival (recessive model: HR: 4.89, 95CI: 5.06 199.70, p<0.01). BIRC5 GC/CC haplotype (rs4789551, rs9904341, and rs8073069) was found in one patient and absent in controls.

Summary/Conclusions: The present study suggests that NFκB2 gene variant (rs12769316, allele A) may be associated with MG susceptibility in males, and BIRC5 (rs9904341) CC genotype may negatively influence MG prognosis. Nonetheless, further studies are needed to validate these findings, enlighten the role of genetic polymorphisms in MG susceptibility and prognosis.

PB1939

SILENCE OF LONG NONCODING RNA MALAT1 BY RNA INTERFERENCE INHIBITS PROLIFERATION AND INDUCES APOPTOSIS IN MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is a neoplastic plasma-cell disorder characterized by abnormal proliferation of monoclonal plasma cells in bone marrow leading to various end-organ damages. Altered long non-coding RNAs (lncRNAs) levels can result in aberrant expression of gene products that may contribute to cancer biology. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), an evolutionarily highly conserved mRNA-like IncRNA was originally identified with high expression in metastatic non-small-cell lung cancer and reported to be up-regulated in many other cancers. However, the function of MALAT1 in MM remains unknown.

Aims: Our study aimed to evaluate the role of MALAT1 on proliferation as well as apoptosis in MM cells in vitro and tumorigenic ability in vivo, following transfection with MALAT1-specific short hairpin RNA (shRNA) expression plasmids.

Methods: Levels of MALAT1 in human myeloma cell lines were detected by real-time polymerase chain reaction (RT-PCR) analysis. The effects of MALAT1 shRNA in MM were investigated in vitro and in vivo.

Results: We found that MALAT1 was high expressing in RPMI8226 and U266 cells. Silencing of MALAT1 by shRNA significantly inhibited the proliferation through cell cycle arrest at G1 phase and induced apoptosis, which was closely associated with activation of caspase-3/9, downregulation of Bcl-2 and upregulation of Bax. Study in vivo revealed that silencing of MALAT1 delayed the tumor growth and led to apoptosis in mice bearing xenograft.

Summary/Conclusions: MALAT1 may serve as a promising novel therapeutic target in human MM. Notably, the inhibition of MALAT1 by shRNA may prove to be an effective genetic therapeutic strategy for MM treatment.

PB1940

LONG NON-CODING RNA MEG3 FUNCTIONS AS A COMPETING ENDORSENCE OF shRNA TO REGULATE PTTEN EXPRESSION BY SPONGING MIR-181A IN MULTIPLE MYELOMA

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Background: Long non-coding RNA maternally expressed gene 3 (MEG3) plays a critical role in cancer progression and metastasis. However, the overall biological role and regulatory mechanism of MEG3 in multiple myeloma (MM) development and progression remains largely unknown.

Aims: To explore the tumour suppression role of IncRNA MEG3 in MM and further reveal the mechanism of MEG3 functions as ceRNA to contribute to MM pathogenesis.

Methods: MEG3 expression was measured in MM patients by real-time PCR.
The effect of MEG3 on cell apoptosis, cell proliferation and angiogenesis were gained from CCK-8, flow cytometric analysis and transwell invasion assays in MM cell lines ARP-1 and LP-1. Insights of the mechanism of competitive endogenous RNA (ceRNA) were gained from bioinformatic analysis, luciferase reporter assays and RNA binding protein immunoprecipitation (RIP) assay.

**Results:** MEG3 expression was significantly decreased in MM patients with advanced tumor stages. MEG3 expression (kappa and lambda) proteins were overexpressed in MEG3-promoted cell apoptosis and inhibited cell proliferation, migration, and angiogenesis in MM ARP1 and LP-1 cell lines. Furthermore, MEG3 increase the expression of phosphatase and tensin homolog (PTEN) and consequently inhibited MM cell proliferation and angiogenesis through sponging miR-181a in the normal serum FLC ratio (ratio < 1.65) analyzed by qRT-PCR.

**Summary/Conclusions:** MEG3 functions as a tumor suppressor in MM. High expression of MEG3 is a marker for good survival. We reveal a novel mechanism that MEG3 as a ceRNA of the PTEN gene by competing for miRNA-181a binding sites and thereby regulate the expression of the PTEN mRNA.

**PB1941**

**IMPROVE RISK-STRATIFICATION OF MULTIPLE MYELOMATOA PATIENTS WITH MICROFLUIDIC DEVICES**

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**Background:** Cytogenetic alterations are required for risk stratification of multiple myeloma (MM); however, current pathology assays performed on bone marrow samples directly can produce false negatives due to the unpredictable distribution and rarity of MM cells. A more accurate method is needed for MM diagnosis and risk-stratification. We develop a new microfluidic device to facilitate CD45 depletion for enhancing the detection of cytogenetic alterations in plasma cells.

**Aims:** Improve accuracy of risk stratification for multiple myeloma patients

**Methods:** Bone marrow samples from 48 MM patients were divided into two parts each. One part was directly detected by classic flow cytometry and FISH while the other part was first enriched by microfluidic size selection and then underwent CD45-depletion (MF-CD45-TACs). The enriched samples were then analyzed by flow cytometry and FISH and compared to the classical analysis.

**Results:** MF-CD45-TACs significantly increased the percentage of CD38+/CD138+ cells to 37.7%±20.4% (P<0.001) compared to 10.3%±6.5% in the unmammalized. After the MF-CD45-TACs enrichment, the detection rate of IgH rearrangement, del(13q14), del(17p) and 1q21 gains rose to 56.3%(P<0.001), 37.5%(P<0.001), 22.9%(P<0.001) and 41.7%(P<0.001), respectively, all significant increases compared to untreated samples.

**Summary/Conclusions:** We have developed a rapid, simple assay for improved diagnostics and risk-stratification for MM. With more precise diagnostics, the clinical outcomes of MM will be significantly improved.

**PB1942**

**SERUM FREE LIGHT CHAIN RATIO IS AN INDEPENDENT RISK FACTOR FOR PROGRESSION IN MONOCYTOLOGICAL GAMMATOPHYTH OF UNDETERMINED SIGNIFICANCE**

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3Background: Monoclonal gammapathy of undetermined significance (MGUS) is a premalignant plasma cell proliferative disorder found in approximately 3% of the general population 50 years of age and older. MGUS is associated with progression to multiple myeloma or related malignancy at a rate of 1% per year. Thus the risk of malignancy for a 50-year-old patient with a 25-year life span is 25%. Aims: We hypothesized that the presence of monoclonal free kappa or lambda immunoglobulin light chains in monoclonal gammapathy of undetermined significance (MGUS), as detected by the serum free light chain (FLC) assay increases the risk of progression to malignancy.

**Methods:** 90 Patients seen at the Hematology consultation from 2010 to 2015 with MGUS have a serum Mproteln less than 30 g/L, bone marrow plasma cell less than 10%, and no anemia, hypercalcemia, lytic bone lesions, or renal failure that would be indicative of a malignant plasma cell disorder. The prognostic effect of abnormal kappa-to-lambda FLC ratio on progression of MGUS was studied. We also examined whether the risk of progression varied depending on the extent to which the FLC ratio was abnormal (the normal serum FLC ratio range of k/λ ratio 0.26 to 1.65).

**Results:** The median age at diagnosis of MGUS was 59 years (35-92years). 62 Womans and 28 Mans Sex ratio=2.2. Serum electrophoresis and immuno electrophoresis or immuno fixation was done in 85 patients. Of these, The median serum M protein size at diagnosis was 12 g/L (1.7-28.5g/L). IgG monoclonal 68 patients (75%), and non IgG monoclonal: 22 patients (25%). A monoclonal light chain was detected in 62 patients, as detected by the serum free light chain (FLC) assay increases the rate of progression to malignancy. An abnormal FLC ratio (kappa-lambda ratio <0.26 or >1.65) was detected in 27 (30%) patients. At a median follow-up of 5 years, malignant progression had occurred in 6 patients (6.6%) with an abnormal serum FLC ratio.

**Summary/Conclusions:** A novel, highly sensitive serum free light chain (FLC) assay is now available for clinical practice. The risk of progression in patients with an abnormal FLC ratio was significantly higher compared with patients with a normal ratio, and was independent of the size and type of the serum monoclonal (M) protein. Overexpression of MEG3 promoted cell apoptosis and inhibited cell proliferation, migration and angiogenesis in MM ARP1 and LP-1 cell lines. Furthermore, MEG3 increase the expression of phosphatase and tensin homolog (PTEN) and consequently inhibit MM cell proliferation and angiogenesis through sponging miR-181a in the normal serum FLC ratio (ratio < 1.65) analyzed by qRT-PCR.

**Aims:** We evaluated the changes of intensity of expression of MDR genes in patients with newly diagnosed and refractory/relapsed multiple myeloma and the effect of expression of MDR genes such as MDR 1, MRP 1, BCRP, LRP on the overall survival of patients after treatment with bortezomib.

**Summary/Conclusions:** The bone marrow of 30 patients (12 men and 18 women) aged 48 to 77 years (median 60 years) with stage III MM by classification Durie-Salmon were studied. 15 patients were included in a newly diagnosed (ND) MM. 15 patients were in group of a clinically refractory/relapsed (RR) MM. The bone marrow in this group of patients were studied after treatment with alkylating agents at the time of registration of resistance to the given therapy. In the future, all patients were treated by bortezomib - containing chemotherapy regimens. mRNA expression studied genes were determined by semi-quantitative polymerase chain reaction reverse transcription. The degree of expression was assessed by semi-quantitative visual assessment from 0 (no photoreetic nuclear bright points (bright points on the nuclei (transcription)) OS was analyzed by the Kaplan-Meier method, with the use of Cox-Mantel test. Differences were considered statistically significant at p<0.05.

**Results:** In both groups of patients had comparable expression of all studied MDR’s genes. The development of clinical resistance to treatment with alkylating agents were accompanied by an increase in mRNA expression of all studied genes. However, the statistically significant increase the expression of the intensity obtained for LRP gene only (the average intensity of the expression of mRNA LRP gene in ND MM 0.9±0.24, with RR MM 1.93±0.34, p<0.05). The MDR 1 mRNA expression was 1.50±0.34 in the group of ND MM and 1.67±0.31 in the group of RR MM, p<0.05. The expression of mRNA of MDR 1 and BCRP are 1.07±0.21 and 1.63±0.15 respectively before treatment and increased to 1.73±0.31 and 2.13±0.35 respectively in the group of RR MM, p=0.06. OS was negatively associated with high LRP gene expression only in group of ND MM (median of OS in patients with high LRP gene expression was 11 months and in those with low expression 62 months, p<0.05).

**Summary/Conclusions:** High expression of LRP gene is associated with worse overall survival in patients with newly diagnosed MM treated with bortezomib-containing chemotherapy programs. "Genetic resource MDR" in MM is due mainly to the initial multidrug resistance. The treatment of MM by alkylating drugs increase the existing at the time of diagnosis of MDR activity of genes.
typing (IL-4, TGF-β1, IL-1α, IL-1β) was performed by PCR-SSP: study of cytokine abnormalities was performed by standard GTG-method and interphase FISH analyses with DNA probes: LSI 13(ER)13q14, IGH/CCND1, IGH/FGFR3, LSI TP53 (17q13.1); p-values less than 0.05 were considered statistically significant.

Results: Previous results allow us to describe some cytokine genotype markers associated with the development of MM (IL-1α -889 TT, IL-1β -3962 TT, IL-6 -1747 GG, IL-6 n565 GG) (gr. 1) as additional negative prognostic markers but IL-4 - 33 CC and TGF-β1 codon 25 GG genotypes as additional positive prognostic markers (gr. 2).

However, in some MM patients we found presence of negative and positive markers together (mixed markers; gr. 3). We analyzed cytokine profiles in MM patients with different prognostic markers in their genotypes (Table 1).

The frequency of abnormal cytogenetic transformations in the 2nd gr. was noticeably lower compared to patients from the 1st and 3rd gr. (0.11 vs 0.78 vs 0.67 respectively; p<0.05). Similarly, significant differences in the frequency between patients with positive prognostic markers and normal cytogenetic profile (0.89) compared to MM patients with negative (0.22) or mixed (0.33) genotypes but normal cytogenetic profiles were also observed (p<0.05). In the 1st gr. frequency of cytogenetic abnormalities was noticeably higher compared to patients with normal profile (0.78 vs 0.22; p<0.05). Vice versa, in patients with positive prognostic markers the frequency of normal cytogenetic profiles was remarkably higher (0.89) compared to patients with aberrations (0.11; p<0.05).

Summary/Conclusions: Thus, our results allow to describe IL-1α -889 TT, IL-1β -3962 TT, IL-6 -1747 GG and IL-6 n565 GG as markers associated with the presence of cytokine abnormalities in MM patient cells. However, IL-4 - 33 CC and TGF-β1 codon 25 GG as additional negative prognostic markers in patients with MM from the North-West region of Russia. Although, if MM patients have both negative and positive prognostic markers associated with the development of multiple myeloma (mixed genotype) it seems that the chance of finding cytokine abnormalities is much higher compared to patients with positive prognostic markers only.

PB1945

CORRELATION DEPENDENCE OF CHRONIC LYMPHOPROLIFERATIVE DISORDERS, MULTIPLE MYELOMA FROM CHANGES OF IMMUNE RESPONSE GENES PROFILE

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Background: Hematological malignancies are multifactorial diseases in the development of which play a role as environmental factors and genetic determinants. The structure of cytokine genes include the presence in human genome of allelic variants of the regulatory regions of the innate immune response genes. At present time, they are considered as real risk factors for these diseases in a person with a certain set of genetic variants. Their distribution among the population corresponds to the population laws and has its ethnographic features. Analysis of the individual associations of genes polymorphic variants involved in the implementation of the immune response does not sufficiently complete answer about their role in the formation of predisposition to the development of chronic lymphoproliferative disorders (CLD) and multiple myeloma (MM). It is noted that in the pathogenesis of hematological diseases these associations contribute significantly to certain combinations of immune response genes.

Aims: Analysis of interactions between genes based on the distribution of immune response genes combinations in chronic lymphoproliferative disorders and multiple myeloma.

Methods: The study included 176 patients aged 22-86 years (median - 61 year), identifying themselves as Caucasians residing in one region in the north-east of the Russian Federation. This group consisted of 80 patients with chronic lymphocytic leukemia/small lymphocytic lymphoma (45%), 72 with multiple myeloma (41%), 10 with diffuse large B-cell lymphoma (6%) with marginal zone lymphoma (3%) four with mantle cell lymphoma (2%), three with lymphoplasmacytic lymphoma (2%) and one patient with follicular lymphoma (1%).

Genotyping of polymorphism of the innate immune response genes TL2R (rs5743708), TL3R (rs3775291), TL6R (rs5743810), TL9R (rs5473836), IL1β (rs2856841), IL2 (rs2069762), IL4 (rs2243250), IL6 (rs1800795), IL10 (rs3082469) (17q11-17q12), IL17A (rs18252279913), CD14 (rs134442490), TNFα (rs3003188), FCGR2A (rs1801274) were performed by polymerase chain reaction with allele-specific primers (LifeTech, Russia). Analysis of interactions between genes was performed using nonparametric GMDR program (Generalized Multifactor-
Myeloma and other monoclonal gammapathies - Clinical

PB1948
Abstract withdrawn.

PB1949
IMPACT OF RENAL IMPAIRMENT IN NEWLY DIAGNOSED MULTIPLE MYELOMA IN A REAL WORLD SETTING
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Background: Renal impairment (RI) is a frequent complication of patients with newly diagnosed multiple myeloma (NDMM), reported in 15-40% with 10% requiring hemodialysis (HD). It is associated with higher early mortality (EM) and lower overall survival (OS). Early diagnosis and treatment with new agents improve these results.

Aims: Analyze renal response, OS and EM in NDMM with RI and compare them to patients with MM without RI.

Methods: All consecutive and unselected NDMM patients treated at Hospital de Clínicas, Montevideo, Uruguay, from January 2011 to June 2015 were included. Our database was completed prospectively and included clinical and laboratory characteristics of the disease, treatment, treatment-related adverse events, response, HD requirement, renal response and mortality.

Diagnosis of MM, response to treatment and degree of renal function recovery were based on the International Myeloma Working Group criteria. RI was defined as an estimated glomerular filtration rate (eGFR) <40 ml/min/1.73m², calculated by MDRD (Modification of Diet in Renal Disease) equation. Patients whose RI was explained by other causes were excluded. Early treatment was defined by initiation within 7 days after diagnosis. EM was defined as death within 3 months of diagnosis.

Results: MM was diagnosed in 52 patients, median age was 67 years (range 39-90), 61.5% were male, 38.5% had RI. The characteristics of the patients and front-line treatment are shown in Figure 1.

PB1950
THE EXPRESSION OF THE TRYP'TASE POSITIVE MAST CELLS AND THE LEVELS OF IL-17, CORRELATE WITH ANGIOGENIC FACTORS IN PATIENTS WITH MULTIPLE MYELOMA
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Background: Angiogenesis in the bone marrow plays a very important role in the progression of multiple myeloma (MM). The procedure of angiogenesis is stimulated by several factors such as VEGF, FGF-2 and metalloproteinases that are secreted straight from the tumor cells. The presence of IL-6 in the microenvironment, induces the production and the secretion of several angiogenic factors that activate inflammatory cells of the matrix, like macrophages and mast cells to secrete more angiogenic factors. IL-17 is among the most important cytokines that have an important role in the development of myeloma tumor. IL-17 is a proinflammatory cytokine that is secreted primarily by CD4+ (activated memory cells) and stimulate macrophages, fibroblasts and other cells that release several cytokines. It has been reported that IL-17, induces angiogenesis in humans by stimulating the migration of vessel endothelial cells and adjusting the production of various proangiogenic factors. In a previous study, it was found that increased levels in stage II and stage III, resolved after therapy. Additionally, blocking the receptor of IL-17, with an antibody, cancels the effects of IL-17.

Aims: Aim of this study is to assess the relationship of the MCD and IL-17, in angiogenesis of MM, as well as their correlation with known angiogenic factors in disease progression.

Methods: We studied 52 newly diagnosed patients with MM, 32 women and 20 men, aged 67.±±9.6 years. According to the ISS stage, 19 were stage I, 17 stage II and 16 stage III. Regarding the type of paraprotein that had been found, 31 IgG, 17 IgA and 4 patients with light chains. 20 age and sex-matched healthy volunteers, were used as controls. Serum samples and bone marrow biopsy samples were obtained from all patients prior to the initiation of treatment. Patients with impaired hepatic and renal function, chronic or acute infections, chronic inflammatory or autoimmune disorders or other malignancies were excluded from the study.We also excluded patients who were taking anti-inflammatory drugs, corticosteroids or bisphosphonates. IL-17, bFGF and ANGIOI-2 were measured in patients’ serum with ELISA method according to the manufacturer’s instructions. The MCD assessed after immunohistochemical staining using monoclonal antibody to mast cell tryptase. The MCD was measured in three hot spots (maximum vasculature area) x 100 and then we measured mast cells, using a graduated slide which corresponds to an area of 0.0625 mm². MCD was calculated as mean MCD / HPF.

Results: Statistically significant differences between patients and controls were observed in all measured parameters, MCD (p < 0.001), bFGF (p < 0.01) and ANGIOI-2 (p < 0.01). All parameters were increased in parallel with ISS stages (p < 0.001) in all cases. Finally, the MCD and IL-17 correlated significantly with all the measured parameters (p < 0.001).

Summary/Conclusions: The mast cells increase in the bone marrow (BM) of patients with MM. They release several transmitters that promote directly and indirectly the development of disease progression of MM also accompanied by increased angiogenesis in BM. In conclusion, mast cells and angiogenic factors seem to be important elements in the development of MM and become potential targets for the treatment and prognosis of the disease.
PB1951
HEALTHCARE RESOURCE UTILIZATION ASSOCIATED WITH DIFFERENT TREATMENT MODALITIES OF RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS IN THE US: FINDINGS FROM PREAMBLE
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Background: Proteasome inhibitors (PIs), immunomodulatory drugs (IMiDs) and treatments involving both a PI and an IMID (PI+IMID) are the principal therapies for treating relapsed/refractory multiple myeloma (RRMM). The widespread adoption of these treatments may come with high healthcare resource utilization (HCRU), of which key drivers are reported in past research. It is important to further understand HCRU by different treatment modalities in real-world practice settings.

Aims: To evaluate HCRU in patients receiving different treatment modalities for RRMM.

Methods: US patients with RRMM, aged ≥18 y, with at least one prior therapy who initiated treatment with a PI, IMID or IMiD+PI within 90 d before or 30 d after study enrollment (index therapy), were identified from PREAMBLE, an ongoing, prospective, multinational, non-interventional observational study. Patient data collected at each healthcare provider (HCP) visit, over a 3-y period or until the end of patient follow-up, included clinic/physician office visits; home healthcare, hospital outpatient and emergency room visits; and hospitalizations. Demographics and baseline characteristics were summarized using descriptive statistics. HCRU and its associated costs were analyzed using a standard per-1000 patients-per-month metric.

Results: 287 patients (median age 66 y; 56% male) were enrolled in the US. At the time of data cut-off (Sep 2016), 136 (47%) were still in the study and 151 (53%) had withdrawn; 92 (61%) of those withdrawn had died. Median (range) follow-up was 12.7 (0.5–4.1) mo. At study entry, patients were divided into three cohorts based on index therapy: PI (n=162, 56%; carfilzomib n=82/162; bortezomib n=80/162), IMID (n=74, 26%; pomalidomide n=32/74; lenalidomide/thalidomide n=42/74), and PI+IMID (n=51, 18%; carfilzomib and/or pomalidomide n=17/51; other n=34/51). The three groups were similar with regard to sex, race, disease status, ISS stage, comorbidities and number of prior therapies (Table 1).

Summary/Conclusions: Routine management of MM and treatment-related events drive HCRU, which may differ by treatment. Hospitalizations and hospital outpatient visits remain key drivers of HCRU in MM, which highlights an unmet medical need for effective therapy with better safety profiles.

PB1952
ASSOCIATION OF SERUM HEAVY/LIGHT CHAIN PAIR SUPPRESSION WITH RISK FACTORS FOR PROGRESSION IN MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE AND SMOLDERING MYELOMA
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Background: Monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM) are conditions that usually precede symptomatic multiple myeloma (MM). Risk stratification is crucial, considering the heterogeneous progression rate among these patients and the chemoprevention trials encouraged for high risk individuals. A number of prognostic factors for progression have been identified. In this sense, the novel Hevylite assay now enables us to accurately measure each isotype-specific heavy and light chain (HLC). Recently, isotype-specific uninvolved HLC pair suppression was described as an independent predictor of progression to MM in patients with MGUS. The role of Hevylite as a prognostic factor in SMM is less investigated.

Aims: The aim of the present study was to analyze the impact of HLC pairs in a series of patients with high risk MGUS and SMM and their relationship with other previously described risk factors.

Methods: Forty-four patients diagnosed with high risk MGUS or SMM at a single institution from March 2014 through April 2016 were prospectively included in the present study. Patients were stratified according to the Mayo Clinic and the Spanish PETHEMA group models. Samples at diagnosis were tested for HLC concentrations for the three pairs (IgG, IgM and IgA) by immunonephelometry.

Results: The clinical characteristics and risk stratification of patients are summarized in Table 1.

Table 1. Patient characteristics and risk stratification.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>Median (IQR)</td>
<td>65 (55-75)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male: 22, Female: 22</td>
<td>50%</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>Median (IQR)</td>
<td>1.5 (1.2-1.8)</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>Median (IQR)</td>
<td>3.9 (3.5-4.2)</td>
</tr>
<tr>
<td>Serum B2M</td>
<td>Median (IQR)</td>
<td>3.5 (2.8-4.2)</td>
</tr>
<tr>
<td>Serum albumin / B2M</td>
<td>Median (IQR)</td>
<td>1.1 (0.9-1.3)</td>
</tr>
<tr>
<td>Serum IgG</td>
<td>Median (IQR)</td>
<td>1.5 (1.2-1.8)</td>
</tr>
<tr>
<td>Serum IgM</td>
<td>Median (IQR)</td>
<td>1.5 (1.2-1.8)</td>
</tr>
<tr>
<td>Serum IgA</td>
<td>Median (IQR)</td>
<td>1.5 (1.2-1.8)</td>
</tr>
<tr>
<td>Serum kappa</td>
<td>Median (IQR)</td>
<td>1.5 (1.2-1.8)</td>
</tr>
<tr>
<td>Serum lambda</td>
<td>Median (IQR)</td>
<td>1.5 (1.2-1.8)</td>
</tr>
<tr>
<td>Serum kappa / lambda</td>
<td>Median (IQR)</td>
<td>1.1 (0.9-1.3)</td>
</tr>
<tr>
<td>Serum kappa + lambda</td>
<td>Median (IQR)</td>
<td>1.5 (1.2-1.8)</td>
</tr>
<tr>
<td>Serum kappa - lambda</td>
<td>Median (IQR)</td>
<td>1.5 (1.2-1.8)</td>
</tr>
<tr>
<td>Serum kappa / lambda</td>
<td>Median (IQR)</td>
<td>1.1 (0.9-1.3)</td>
</tr>
</tbody>
</table>

An abnormal HLC-pair ratio was detected in 96% of MGUS and 94% of SMM patients, with no differences depending on the heavy chain isotype. A highly abnormal HLC ratio (<0.02 or >45) was present in 9 patients (1 with MGUS and 8 with SMM). HLC-pair suppression (i.e., IgG-x in patients with IgG-x gammapathy) was more frequent in patients with SMM (83% vs 46%, p=0.02). Severe HLC-pair suppression (>50% below lower level of normal) was present in 12 (27%) patients, the majority of which had a diagnosis of SMM (83%). Severe HLC-pair suppression was significantly associated with a highly abnormal (<0.125 or >8) serum free light chain (FLC) ratio (p=0.004), abnormal/normal bone marrow plasma cell ratio >0.95 (p=0.001) and immunomaropao (p=0.005), being present in 6 (86%) of the 7 patients with high risk SMM. Suppression of the other isotypes (i.e., IgA or IgM HLC pairs in a patient with IgG gammapathy) was identified in 33 (75%) patients, namely in 18 (69%) patients with MGUS and 15 (83%) patients with SMM (p=0.48), and was not significantly higher in the SMM group.
associated with other risk factors for progression. Severe suppression (>50% lower level of normal) was significantly more frequent in sEMD patients (33% vs 8%, p=0.04) and was associated with highly abnormal FLC ratio (p<0.001), abnormal/normal plasma cell ratio >0.95 (p<0.001), severe HLC-pair suppression (p<0.001) and highly abnormal HLC ratio at diagnosis (p<0.005). The “evolving” pattern of the serum M-protein was identified in 12 patients (15%) and it was significantly associated with either severe suppression of the HLC-pair or of the other isotypes. After a median follow-up of 18 months (range, 6-35) progression to symptomatic MM was observed in 3 patients. All 3 had a diagnosis of SMM with an “evolving” pattern, highly abnormal HLC-ratio and severe HLC-pair suppression.

Summary/Conclusions: The findings presented in this study indicate that highly abnormal HLC ratio, severe suppression of the HLC-matched pair and other isotype HLC pairs are associated with known risk factors for disease progression in patients with high risk MGUS and SMM. The HLC assay could become a valuable tool in the risk stratification of these patients.

PB1953
EXTRAMEDULLARY MYELOMA IN THE “NOVEL AGENTS ERA”: OUTCOME, HETEROGENEITIES AND PECULIARITIES OF A COHORT OF 84 PATIENTS RETROSPECTIVELY ANALYSED IN A MONOCENTRIC EXPERIENCE
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Background: Extradary myeloma is an uncommon manifestation in multiple myeloma (MM) and can either accompany newly diagnosed disease or develop with disease progression or relapse. Extradary myeloma (EMM) seems to have a different pathogenesis from its much more frequently encountered medullary counterpart, showing often a poor prognosis. EMM clinical situations are extraordinarily heterogeneous and their management is challenging. This includes organ or tissue involvement resulting from hematogenous-spread and/or bone involvement originating from different kind of bones.

Aims: We aimed to evaluate the impact of these diseases on patients’ outcome in the context of novel-agents.

Methods: We reviewed patients presenting EMM (median age 60, range 30-76) describing clinical and biological features (Figure 1B. Our aim was studying prognosis of bone-related extradary disease (bEMD) and its relationship with soft-tissue related EMM (sEMD) in MM patients in our institution.

Results: 42 bEMD and 42 sEMD patients treated at Our Department between 2007 and 2016 were included in this study. Of the first group 10 patients presented EMM at diagnosis and 32 at relapse as well as 7 and 35 respectively of the second series. 31 among sEMD were dead and 11 were alive, 20 of bEMD patients were dead and 22 were still alive. EM was diagnosed using imaging techniques such as PET-CT (35%) or magnetic resonance MRI (65%). Biopsy showed tumor in all patients (97%). The treatment was heterogeneous and all patients had received either thalidomide or bortezomib in the first-line of therapy. We showed that sEMD cohort has a significantly poorer survival compared to bEMD patients (median OS from diagnosis of EMM of 13 versus 58 months, P<0.001). Finally lung, liver (parenchyma-EM) and lymph nodes were involved in 33%, 37% and 14% of sEMD patients, respectively. In sEMD patients a better outcome was observed in skin and lymph nodes involvement (sMM 50% vs bEMD 75% of OS, respectively). When compared to skin and lymph nodes masses respectively median OS of 12 and 10 months versus 18 and 15 months P <0.001). Conversely among bEMD group there wasn’t a significant advantage of outcome regarding the different bones involved. Kaplan-Meier estimates were used for survival analysis and differences between survival-times in patient subgroups were tested using the log-rank test (Figure 1A). Interestingly extramedullary-spread can be triggered by an invasive-procedures (surgery) or by a bone-fraction. In our population we have a case of breast-plasmocytoma diagnosed accidentally after reconstructive breast-surgery, where Polymerase Chain Reaction of immunoglobulin gene rearrangement in the breast tissue excised confirmed the diagnosis.

Conclusions: The extramedullary-myeloma disease is an uncommon manifestation in multiple myeloma and it can be seen at different stages of disease: newly diagnosed, relapse, progression. In our experience EMM was detected at diagnosis in 31% of patients and 32% at relapse.

PB1954
DINAMIC PREDICTIVE FACTORS FOR A BETTER STRATIFICATION OF PATIENTS WITH R-ISS II NEWLY DIAGNOSED MULTIPLE MYELOMA
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Background: Revised International Staging System (R-ISS), combining the ISS score with cytogenetics and serum LDH, represents the most recent prognostic model for stratifying newly diagnosed multiple myeloma patients into three different survival groups. Although data for R-ISS development have been obtained from patients enrolled in clinical trials, this prognostic score has been validated also in real-life scenario (Tandon et al., 2017). In both non-clinical trial setting and IMWG experience, the majority of patients (about 65%) belonged to the intermediate risk group (R-ISS II) that, probably, needs better prognostication.

Aims: The aim of this study was to search for a closer stratification of MM patients with R-ISS II, taking into consideration dynamic aspects, such as therapeutic strategy and response to therapy.

Methods: We investigated the impact of variables, such as initial therapy, response to therapy and maintenance therapy, on PFS and OS in 108 newly diagnosed MM patients classified as R-ISS stage II, diagnosed between 2005 and 2015, who received novel agents such as immunomodulatory drugs and proteasome inhibitors. Score weights of the prognostic factors, found to be significant according to Cox regression model, were determined based on the regression coefficients.

Results: Median age of the 108 patients was 69 years (range 44-93) and 35% of them were older than 75 years. Thalidomide- and lenalidomide-based regimens were administered to 12% and 28% of patients, respectively, whereas 60% of the patients received bortezomib (54%) or carfilzomib-based (6%) regimens as induction therapy. Thirty-eight percent of the study population underwent ASCT and 40% received maintenance therapy. Regarding the response to the therapy, at least CR, VGPR and PR were documented in 35%, 66% and 87% of 108 patients respectively. Five-year PFS and OS were 31% and 65%, respectively, similar to those reported by IMWG. Patients who did not achieve a CR, showed a significantly shorter 5yr-PFS (27% vs 50%; HR=2.9, 95%Ci=1.6-4.50; p<0.0001) and 5yr-OS (53% vs 80%; HR=2.8, 95%Ci=1.3-5.9; p=0.006) compared to those who did. Moreover, a significant better 5yr-PFS was observed in patients receiving maintenance therapy, compared to those who did not receive maintenance therapy (48% vs 20%; HR=1.9, 95%CI=1.2-3.3; p=0.010) whereas initial therapy did not affect the outcome. Assigning a value to the variables found to be significantly related to survival measures, according to the above methods, patients were stratified into the following two groups: low-risk (LR), including 36 patients with score 0-1, i.e. patients achieving CR and receiving maintenance therapy (score 0) or achieving CR but not receiving maintenance therapy (score 1); high-risk (HR) group, including 72 patients with score 2-3, i.e. not achieving CR, who underwent maintenance therapy (score 2) or not achieving CR and not receiving maintenance therapy (score 3). Five-year PFS of HR patients was significantly shorter (HR=1.9, 95%CI=1.6-3.8; p<0.0001), whereas 5-year OS was 57% vs 80% (HR=1.9, 95%CI=1.1-3.3; p=0.021).

Summary/Conclusions: Our results suggest that in the R-ISS II MM patients,
the outcome of those achieving a CR and undergoing long-term therapy, is comparable with the outcome of the R-ISS I group. On the other hand, patients not achieving CR have a poor outcome, similar to those in the R-ISS III group. Therefore, these patients should require personalized therapy, aimed to achieve CR and to maintain therapy continuously.

PB1955

THE IMPACT OF THE UPDATED IMWG DIAGNOSTIC CRITERIA IN A REAL-LIFE SMM COHORT: A SINGLE CENTER EXPERIENCE
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Background: Recently, an update of the diagnostic criteria for smoldering multiple myeloma (SMM) & multiple myeloma (MM) was published by the InternationaMyeloma Working Group (IMWG). In addition to CRAB criteria, 3 biomarkers of disease were introduced being (i) the presence of >60% clonal bone marrow plasma cells (BMPC), (ii) a serum free light chain ratio (FLC-ratio) >100 & (iii) the presence of ≥1 focal lesion on whole-body MRI (WBMRI). The introduction of these biomarkers has been identified to identify patients having a 70-80% risk of progression to MM over a 2-year time period.

Aims: To evaluate the impact of IMWG criteria in routine practice, focussing on (i) the prevalence of these biomarkers, (ii) the diagnostic strength of BMPC estimation, respectively (FLC-ratio) & (iii) the added role of dynamic contrast-enhanced WBMRI (DCEMRI) in the evaluation of SMM patients.

Methods: We retrospectively identified 28 SMM cases diagnosed between 01/01-09/31/12/14. Sufficient data for analysis was available for 25 patients. All patients underwent standard clinical & laboratory evaluation, bone marrow examination & WBMRI (T1- (+/-Gd) & T2-weighted sequences, diffusion-weighted sequences & additional DCEMRI sequences using time intensity curves). Time to progression (TTP) is defined as time from diagnosis until MM development. Overall survival (OS) is defined as time from diagnosis until death from any cause. Survival analysis was performed using the Kaplan-Meier method & significance was tested using the log-rank algorithm. Intergroup analysis was performed using non-parametric rank-based analysis & correlation was calculated using the Pearson coefficient. Reported p-values are 2-sided with a significance level of 5%.

Results: Median follow-up was 64.1 months (analysis performed on 01/02/2017). No patients had a FLC-ratio >100 at time of diagnosis. Also, no patients with >60% of clonal BMPCs were seen. In 20 patients BMPC counts using both asparate & biopsy were available. Analysis showed a significant higher estimate of BMPC levels using biopsy (14.8%, SD 4.99) versus aspirate (6.45%, SD 6.59) (p=0.02). Sensitivity of bone marrow aspirate was calculated to be 30% considering the 10% BMPC cut-off. Correlation between bone marrow aspirate & biopsy was found in 26.2% of cases. WBMRI-positivity was seen in 9 patients (36%). Progression was seen in 7/9 patients (78%) where only 1/16 WBMRI-negative patients (6.3%) developed MM (p=0.001). Median TTP was 19.9 months versus not reached (p=0.001). No correlation was seen of dynamic contrast-enhanced WBMRI between both groups (p=0.453). DCEMRI was positive in 14 patients (56%) thus identifying 5 additional WBMRI-negative patients with measurable bone marrow involvement. No significant difference concerning progression risk was however seen between WBMRI-positive patients being DCEMRI-positive (5/19, 26.3%) or -negative (14/19, 73.7%) (p=0.317). Median follow-back of patients was revealed no difference concerning age, sex, genetic aberrations or the type of the monoclonal protein between both groups. In patients developing MM, progression was seen based on the development of anemia (5/8, 62.5%), bone pain (3/8, 37.5%), hypercalcemia (1/8, 12.5%) & the development of punched-out lesions (4/8, 50%). No renal insufficiencies were observed.

Summary/Conclusions: Our data shows that WBMRI-positivity was the most frequent biomarker in a routine clinical setting. WBMRI-positivity, according to IMWG-criteria, clearly identifies patients with an increased risk of progression as was already shown previously. Although increasing the sensitivity of WBMRI, addition of DCEMRI-sequences didn’t have an added benefit. Our sample size was however relatively small. And although IMWG-guidelines do not state clear requirements concerning the preferred type of bone marrow evaluation, our data shows that a bone marrow biopsy can never be omitted in suspected cases of SMM, as an aspirate alone clearly lacks diagnostic strength.

PB1956

RISK FACTORS FOR VENOUS THROMBOEMBOLISM IN 401 MULTIPLE MYELOMA PATIENTS: OBSERVATION OVER A 25-YEARS PERIOD IN A SINGLE INSTITUTION
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Background: Patients with multiple myeloma (MM) have shown an incidence of 3-10% of venous thromboembolic events (VTE). The introduction of immunomodulatory drugs (IMiDs) in the treatment regimen has further increased the risk of VTE, especially when combined with steroids or chemotherapy (20-30%). Actual guidelines recommend thromboprophylaxis measures, but the proposed strategies are the results of expertise consensus or derived from the extrapolation of data from many studies.

Aims: The aim of this study is to analyze the development of VTE in a large cohort of MM patients, treated for 25 years in a single institution, to assess risk factors indicated in general population, actors suggested VTE risk population, also to confirm the relationship of risk of IMiDs-based regimens and the relevance of anticoagulant thromboprophylaxis.

Methods: Four hundred and one consecutive patients diagnosed with MM in a tertiary University Hospital between 1991 to 2015 were included. Data about VTE development, patient characteristics, myeloma-related factors, treatment and thromboprophylactic measures were retrospectively recorded. Multivariable correlates of VTE were assessed using Cox proportional hazard analyses.

Results: The median age at diagnosis was 68 years (range 24-90 years), and 47% were males. The results concerning treatment are extracted from 374 patients who were symptomatically received myeloma diagnosis, among the 164 patients that received IMiDs-based regimen, 27% did not receive any antithrombotic treatment, due to the lack of strong recommendations at the beginning of the use of IMiDs-based regimens. On the other hand, the most common thromboprophylaxis was set with LMWH (124/345, following by low doses of aspirin (13%) and anti-vitamin K (VKA) (8%). Median follow was 40 months (range, 1-293) and VTE occurred in 11% of patients, with a medium time from diagnosis of 10 months. IMiDs based-regimen demonstrated to be a risk factor associated on multivariate analysis, and the relevance of thromboprophylaxis has been proved, as the absence of this measure increased significantly the risk of VTE, other factors that have also demonstrated to be independently associated with a higher risk for VTE were: BMI ≥30 Kg/m2, prior Stroke or TIA, prior malignancy neoplasm, and the use of high dose of dexamethasone.

Summary/Conclusions: Our data support the actual recommendation of antithrombotic prophylaxis in IMiDs-based regimens, especially in association with high dose of dexamethasone. We recommend the use of a risk factor model including obesity and previous history of thromboembolic disease or cancer, in order to guide the appropriate thromboprophylaxis measures.

PB1957

A PHASE III RANDOMIZED, OPEN-LABEL STUDY OF ISATUXIMAB (SAR650984) PLUS POMALIDOMIDE AND DEXAMETHASONE VERSUS POM AND DEX IN RELAPSED/REFRACTORY MULTIPLE MYELOMA
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Summary/Conclusions: This Phase III, prospective, multicenter, randomized, open-label study compared the efficacy and safety of ISA+Pom/dex versus Pom/dex in the treatment of adult patients with RRMM. Eligible patients are those with RRMM and demonstrated disease progression within 60 days of the last therapy. Patients will have received at least 2 prior lines of therapy, including lenalidomide and a proteasome inhibitor (bortezomib, carfilzomib, or ixazomib) alone or in combination. Patients will be randomly assigned in a 1:1 ratio to either ISA (10 mg/kg IV on Days 1, 8, 15, and 22 in the 1st cycle; Days 1 and 15 in subsequent cycles) plus Pom (4 mg on Days 1–21) and dex (at 40 mg for patients <75 years of age and at 20 mg for patients ≥75 years of age for 28 days) or Pom and dex. Treatment cycles will be 28 days each. Patients will continue therapy until disease progression, occurrence of unacceptable adverse events (AEs), or their decision to discontinue the study, whichever comes first. All patients will be provided informed consent. The primary endpoint is progression-free survival (PFS), i.e. time from randomization to progression, death, or any cause. Response will be determined by IMWG criteria (2016). Key secondary endpoints include overall response rate and overall survival (OS). Safety evaluations include treatment-emergent AEs/serious AEs (including infusion-associated reactions), laboratory parameters, vital signs, and assessment of physical examination.

Results: Approximately 300 patients (150 in each arm) are expected to be enrolled in this study. Statistical analyses will be conducted according to a pre-specified plan. The first patient was recruited in January 2017.

Summary/Conclusions: This Phase III, prospective, multicenter trial will provide preliminary data on the safety and efficacy of ISA+Pom/dex versus Pom/dex, a combination which has previously reported preliminary clinical activity and manageable toxicities in heavily pretreated patients with RRMM in a single-arm Phase Ib study.
LONG TERM SURVIVAL OF IGM MULTIPLE MYELOMA AND WALDENSTROM’S MACROGLOBULINEMIA PATIENTS

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Background: IgM multiple myeloma (MM) and Waldenström’s macroglobulinemia (WM) are two hematologic malignancies with the common finding of IgM monoclonal gammopathy. IgM MM is a rare and poorly characterized disease.

Aims: The paper presents clinical and laboratory results of long term observations of 15 IgM MM patients selected from a group of 889 MM patients (1.6%) diagnosed and treated for several years at the Institute of Hematology and Transfusion Medicine in Warsaw as well as 15 WM patients investigated and treated at the same period of time at our hospital.

Methods: For analysis of serum proteins new Hevylite and Freelite tests (Binding Site Ltd Birmingham, UK) were applied as well as immunofixation using Sebia (Lisses, France) reagents. Fresh and archived frozen serum samples were used for the study.

Results: The clinical presentation of IgM MM patients is heterogenic starting with typical form for non IgM MM through predominant form with characteristic hyperviscosity syndrome and severe disease course to slow and latent form with survival time up to dozens of years. In 2 patients diagnosis of IgM MM was preceded by a 3-year period of monoclonal gammopathy ofundetermined significance (MGUS) while in 4 patients (27%) diagnosis of WM was preceded by a 108, 84, 78, 9 months period of IgM MGUS. Median real overall survival of IgM MM patients was 50 months, 5 patients (33%) survived above 7 years and 2 patients (13%) survived above 12 years. Median survival of WM patients was 108 months, 7 patients (47%) survived above 10 years, 3 patients (20%) survived above 15 years. Lytic bone lesions were found in 11 (73%) IgM MM patients and in 3 (20%) WM patients. Urine monoclonal free light chains (FLC) detected by immunofixation was present in 60% of IgM MM patients and in 3 (20%) WM patients. Urine monoclonal free light chains (FLC) detected by immunofixation was present in 60% of IgM MM patients and in 3 (20%) WM patients.

Summary/Conclusions: 33% of IgM MM patients survive above 7 years and 13% above 12 years while 47% of WM patients survive above 10 years and 24% above 15 years. Suppression of uninvolved polyclonal IgM (detectable by using HLC test) at the time of IgM myeloma diagnosis is unfavorable prognostic factor.

PRACTICE GAPS AND BARRIERS TO OPTIMAL MANAGEMENT OF MULTIPLE MYELOMA PATIENTS: RESULTS FROM A MIXED-METHODS STUDY IN 8 EUROPEAN COUNTRIES

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Background: Previous studies have identified gaps and barriers in Multiple Myeloma (MM) patient care, especially in relation to treatment decision making. The aim of this study was to get better understanding the practice gaps, from the healthcare providers’ perspectives, with the purpose to investigate the root causes of those gaps and find solutions to alleviate the challenges.

Aims: We conducted a study to identify the practice gaps and challenges in the diagnosis, treatment and management of MM patients, as experienced and reported by medical oncologists, haematologists and haematology-oncologists (HEM) and oncology nurses (NU) in 8 European countries for the treatment of MM.

Methods: This mixed methods ethics-approved study included exploratory semi-structured interviews (phase 1) designed to generate in-depth discussion of the challenges in the diagnosis, treatment and management of MM, followed by a quantitative online survey (phase 2) designed to validate the findings from the interviews with a larger sample. Practice gaps were identified through combined analysis of data from the in-depth interviews and online surveys.

Results: A total of 364 participants (HEM=281, NU=83) from France (n=58), Germany (n=58), Russia (n=41), Spain (n=58), Italy (n=50), the UK (n=58), the Netherlands (n=16), and Belgium (n=25) participated in this study. Thirty-nine (39) interviews were conducted (HEM=28, NU=11) and 325 participants completed the online survey (HEM=253, NU=72). A majority (79%) of the sample had more than 10 years of clinical practice experience and over a third (39%) had over 20% of MM patients in their patient caseload. Three key findings were identified in the management of MM patients: 1) challenges in managing treatment side-effects. Forty percent (40%) of HEM reported lack of skills in managing cardiovascular side effects or symptoms. Over a third of HEM reported difficulties in managing fatigue (40%), skin toxicities (35%) or peripheral neuropathy (34%). NU reported difficulties in understanding the practice gaps, from the healthcare providers’ perspectives, with the purpose to investigate the root causes of those gaps and find solutions to alleviate the challenges.

Summary/Conclusions: These findings provide real-life recent evidence of the challenges of HEM and NU in relation to specific aspects of the management of patients with MM with 3 main areas, challenges in managing side effects, communication with patients and leverage of guidelines which show differences between HEM and NU but also between countries. The findings can be a great input for the development of tailored clinical tools, educational activities and performance improvement interventions, adapted to the local context at a country level. Efforts should aim to address those current challenges before new therapies, such as immunotherapies, become available.
Background: Multiple myeloma (MM) is a malignant proliferation of plasma cells and is characterized by the accumulation of monoclonal plasma cells in bone marrow that secrete pathological monoclonal immunoglobulins. Induc- tive factors secreted by tumor cells and other cells of the marrow microenvironment play an important role in disease progression. APRIL, by initial letters A Proliferation Inducing Ligand, is a member of the family of pro TNF, one of the main factors for the survival of immature and activated B cells. One of the main signal transduction pathways evoked as the percentage of neoplastic plasma cells staining was observed at the other cellular components of BM. The degree of staining expression was evaluated as the percentage of neoplastic plasma cells in BM biopsy samples was obtained from all patients prior to the initiation of treatment. Patients with impaired hepatic and renal function, chronic or acute infections, chronic inflammatory or autoimmune disorders or other malignancies were excluded from the study. We also excluded patients who were taking anti-inflammatory drugs, corticosteroids or bisphosphonates. 20 age and sex-matched healthy volunteers, were used as controls. The levels of IL-6 and IL-10 in the serum were measured by ELIZA. Bone marrow infiltration by neoplastic plasma cells was calculated in%. The expression of cell proliferation index was calculated in BM biopsy sections with immunohistochemistry techniques. The expression of APRIL was also calculated with immunohistochemistry. For the control of the process we used positive control. The assessing of the staining was checked in the optical microscope, over the whole surface of each sample and had to do with the cytoplasms of tumor cells. It was dotted with brown ting. Non-specific staining was observed at the other cellular components of BM. The degree of staining expression was evaluated as the percentage of neoplastic plasma cells and according to the intensity of staining in four-grade scale 0: negative, +1 weak, +2 +3 intense staining. Then the proportion of plasma cells stained for each type of staining separately, was calculated using the H-score method (Histoscore), based on the formula: % *% * 1+2+3%. * Our aim is to prove if the intensity of expression is associated with disease stage.

Results: Statistically significant differences were observed between patients and controls for all parameters measured (p<0.001 in all cases). All values of the measured parameters increased in parallel with the ISS stages of the disease. BMI infiltration ≥ 0.003 Ki-67 ≥0.01, IL-10 <0.001, IL-6, p<0.001). Eventually APRIL correlated significantly with all measured parameters e.g. BMI infiltration =0.386, p<0.01, with Ki-67 =0.390 p <0.10 IL-10 =0.497 p <0.001, IL-6, r=0.484 p <0.001.

Summary/Conclusions: Increased expression of APRIL ligand plays an important role in development and pathobiology of MM and may be an important therapeutic target in the treatment of MM.

References
Risk groups were defined based on the overall score. To provide optimal patient care, each OS predictor were multiplied to obtain an overall score for each patient. Although a tendency towards a higher PFE was observed in the EMP group, it was not statistically significant. No differences were found in PFS/OS between age groups (<60 or ≥60 years), axial vs appendicular skeleton location in SPB, type of treatment received, or the presence of MB. Furthermore, no association was found between the presence of MB at diagnosis and progression to MM (Figure 1).

Figure 1.

Summary/Conclusions: The age at diagnosis of SPB is significantly lower than EMP. Moreover, the progression to MM is notably higher in this group of patients. These distinct characteristics in clinical presentation and outcome could suggest a biological difference between both entities.

PB1964
RISK STRATIFICATION ALGORITHM USING REAL-WORLD DATA FROM PATIENTS WITH RELAPSED AND/OR REFRACTORY MULTIPLE MYELOMA: DESCRIPTION OF CLINICAL OUTCOME BY TREATMENT REGIMEN
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Background: Estimation of survival for patients with RRMM, requires prognostic tools that define the relative risk of death after first relapse. We recently developed a risk stratification algorithm (RSA) using real-world data from the Czech Registry of Monoclonal Gammopathies (RMG). Our RSA uses patient and disease characteristics at diagnosis and at initiation of second-line treatment (2L), and previous treatment outcomes to stratify patients based on their overall survival (OS) expectations from initiation of 2L treatment (Hajek et al. Blood 2016). The value of such an algorithm depends on its validation, but also on understanding the evidence that explains these differences in survival expectations.

Aims: To describe 2L treatment patterns by RSA group and to report OS, progression-free survival (PFS) and response by treatment received in 2L per RSA risk group.

Methods: Data were collected from the Czech RMG for patients aged ≥18 years who were diagnosed with symptomatic MM between May 2007 and April 2016 and in whom 2L treatment had been initiated. Predictors of OS from the start of 2L were identified using Cox regression analyses. Hazard ratios for each OS predictor were multiplied to obtain an overall score for each patient. Risk groups were defined based on the overall score. To provide optimal patient stratification, cut-offs of the score were estimated using K-adaptive partitioning for survival (KAPS) analysis.

Results: Data from 1418 patients were analysed. KAPS analysis defined four groups based on risk of death: low (LR; score ≤ 4.1; n=403), intermediate-low (ILR; score 4.2–10.3; n=635), intermediate-high (IHR; score 10.4–20.1; n=237) and high (HR; score ≥20.2; n=143) risk. Median OS (months) was 57, 29, 13 and 5 for the LR, ILR, IHR and HR groups, respectively. Following stratification, compared with patients in the lower risk groups, a higher proportion of those in the HR group had LDH levels above 360 U/L and an Eastern Cooperative Oncology Group Performance Status of 3–4 at initiation of 2L. Treatments received at 2L were similar across all risk groups, with bortezomib and lenalidomide being the most common 2L treatments. Patients who received bortezomib at 1L were often given lenalidomide or thalidomide at 2L and those who received thalidomide at 1L were frequently given bortezomib at 2L. This suggests that 2L treatment choice was not defined by the underlying risk of death for each patient, but rather by the type of previous treatment. For patients receiving lenalidomide at 2L (months) from start of 2L was 57, 29, 13 and 6 (Figure 1), and median PFS (months) was 18, 12, 8 and 3 in the LR, ILR, IHR and HR groups, respectively. A very good partial response or better (VGPR+) was reported for 29.3%, 31.0%, 18.7% and 16.9% of patients in the LR, ILR, IHR and HR groups, respectively. For patients receiving lenalidomide at 2L, median OS (months) was 45, 29, 14 and 5, and median PFS (months) was 20, 12, 10 and 3 for patients in the LR, ILR, IHR and HR groups, respectively. A VGPR+ was reported for 33.6%, 22.9%, 26.0% and 7.1% of patients in the LR, ILR, IHR and HR groups, respectively.

Figure 1.

Summary/Conclusions: The RSA effectively stratifies patients according to OS from initiation of 2L. However, these results must be validated in an external dataset. The outcomes of each risk group are mainly driven by the underlying risk of death at initiation of 2L; treatment with bortezomib or lenalidomide provided similar outcomes independent of risk group. Use of our RSA at 2L would support physician decision making to improve patient specific care.

PB1965
LACK OF CD56 Expression in Myeloma Cells Decreases the Adherence of Myeloma Cells to the Cell Matrix and is Associated with Poor Prognosis
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Background: Multiple myeloma (MM) is a hematologic disease in which accumulation of malignant plasma cells and high levels of monoclonal protein and free light chains lead to bone marrow failure, hypercalcemia, lytic bone lesions and renal failure. Myeloma cells are distinguished from normal plasma cells by an aberrant immunophenotype. They express CD56 at a level in 70–80% and can be used to distinguish myeloma cells by flow cytometry. The expression of CD56 is constant throughout the course of the disease. The lack of CD56 expression in myeloma cells decreases the adherence of myeloma cells to the cell matrix and is associated with higher levels of bone marrow infiltration and peripheral blood involvement, higher incidence of extramedullary disease, renal insufficiency, Bence Jones protein, plasma cell leukemia and t(11;14). The lack of CD117 expression is associated with poor prognosis of patients with multiple myeloma. The objective of the present study was to evaluate the expression of CD56 in 132 multiple myeloma patients to confirm previous findings of decreased CD56 expression in myeloma cells and to detect its possible association with clinical and laboratory parameters of myeloma disease.
aberrations including t(11;14), t(4;14) and del(13q). CD28 expression is present in 15–45% of patients and is associated with unfavorable cytogenetic changes including t(4;14) and del(17p) and shorter PFS and OS despite aHSCT.

**Aims:** Aim of our retrospective study was to evaluate the impact of CD56, CD117 and CD28 expression on clinical characteristics and PFS in newly diagnosed MM patients treated with bortezomib based induction therapy.

**Methods:** We retrospectively analyzed 110 newly diagnosed MM patients from our national registry that had data available at the time of diagnosis. Immunophenotype was determined using a panel consisting of CD19/CD38/CD45/CD56/CD138 to distinguish and to enumerate MM cells. Monoclonal antibodies directed against CD20, CD28, and CD117 were used additionally. All samples were routinely tested for the presence of recurrent chromosomal aberrations, i.e. del 1p, amp1q, del6q, amp15q, del13, del17, t(4;14), t(14;16) and t(11;14) using commercially available DNA probes.

**Results:** We found no association between CD56 expression and age, gender, elevated LDH or RISS stage. We found a strong association between lack of CD56 expression and light-chain only or asymptomatic myeloma. There was an association between CD28 expression and female gender (Table 1). In multivariate analysis including age, elevated creatinine, RISS, aHSCT, CD28, CD56 and CD117 expression, CD56 expression was associated with a 47% reduced hazard for progression (Exp(B)=0.527, p=0.03). Other factors with statistically significant impact on progression were aHSCT and age. In patients not undergoing aHSCT lacking CD56 expression in comparison to those with an aberrant CD56 expression, the difference in PFS was statistically significant with a PFS of 8 vs 18 Month (Log Rank p=0.088, Breslow p=0.046). When stratified according to RISS stage, only patients in stage 2 disease had a significant reduction in PFS with lack of CD56 expression.

**Summary/Conclusions:** CD56 expression was a prognostic factor for PFS only in the patient cohort not undergoing aHSCT. As previously reported aHSCT seems to abrogate the negative impact of CD56 negativity. We propose CD56 expression to be used as a prognostic marker in patients with RISS stage 2 disease and to use these patients should undergo aHSCT.

**PB1966**

**AUTOLOGOUS TRANSPLANTATION FOR MULTIPLE MYELOMA IN GERMANY – REAL-WORLD DATA FROM A NATIONWIDE, MULTI-INSTITUTIONAL SURVEY IN 2015-2016**

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**Background:** Myeloma is an incurable plasma cell malignancy, in which aggressive regimens may require salvage cytotoxic infusional chemotherapy. Several clinical trials demonstrating the efficacy of bortezomib led to institutional practice changes where vincristine was replaced with bortezomib in the modified hyperCVAD (mod-CVAD) regimen, creating a new treatment regimen, ‘bortezomib-hyperCAD’ (bort-CVAD).

**Aims:** The primary objective is to describe the safety and efficacy of the hyper-CVAD regimen with vincristine or bortezomib in patients with relapsed or refractory MM treated at Oregon Health and Science University.

**Methods:** IRB approval was obtained to perform this retrospective analysis. We describe the effectiveness and tolerability of the 2 regimens among 33 patients with relapsed and/or refractory multiple myeloma (RRMM). Patients who received 21 cycle of mod-CVAD (n=15) or bort-CVAD (n=18) from Jan 1 2011 and Dec 31 2015 at the Knight Cancer Institute were included. Most patients were previously treated with/refractory to proteasome inhibitors (97%/86%) respectively. 13 received prior autologous stem cell transplant (auto-HCT), the median number of prior lines was 3 (range 1-8). High risk cytogenetic factors t(4;14), t(14;16), or del 17p were present in 8 and extramedullary disease in 13 patients overall.

Regimens contained cyclophosphamide 300 mg/m2 IV every 12 hours for 8 doses; doxorubicin 9 mg/m2/day continuous IV infusion every 24 hours and dexamethasone 40 mg by mouth on days 1-4; vincristine 0.4 mg/continuous IV infusion every 24 hours on days 1-4 (mod-CVAD) or bortezomib 1.3mg/m2 SQ on day 1 and 4 (bort-CVAD). All patients received MESNA 350 mg/m2 IV every 24 hours on days 1 through 4; granulocyte colony-stimulating factor 24-48 hours following the completion of chemotherapy; and standard infectious prophylaxis. International Myeloma Working Group uniform response and European Society for Blood and Marrow for minor response (MR) criteria were used.

**Results:** The median number of cycles given was 2 (range 1-6). Cycles were repeated every 3 to 4 weeks. Median follow up was 48 and 33 months in mod-CVAD and bort-CVAD respectively. The ORR was 36% in the mod-CVAD group: 6 partial (PR), 6 minor (MR), and 3 stable disease (SD) compared to 44.4% in the bort-CVAD group: 1 complete response, 7 PR, 2 MR, 6 SD and 2 progressive disease (Fisher’s exact p=0.80). A total of 13 patients proceeded to autologous stem cell transplantation (5 in mod-CVAD and 8 in bort-CVAD respectively). There was no statistically significant association between treatment and febrile neutropenia (29% vs 32%), hospitalizations (29% vs 31%), transfusions (23% vs 24%), and other events. Median number of cycles given and duration of follow-up were similar between the two groups. However, there was a statistically significant difference in safety and tolerability between treatment arms. Three and 2 patients in the mod-CVAD and bort-CVAD arms respectively experienced a major decrease in platelet transfusions.

**Conclusions:** Overall effectiveness and safety outcomes were similar between mod-CVAD and bort-CVAD, with both regimens demonstrating an impressive response rate among heavily pre-treated patients with relapsed/refractory disease. This is a useful salvage strategy to gain rapid dis...
ease control; and as a bridge to other therapies including stem cell transplant and novel therapies.

**PB1968**

**EFFICACY AND SAFETY OF LENALIDOMIDE AND DEXAMETHASONE IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA: A REAL LIFE EXPERIENCE FROM TURKEY**

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**Background:** Lenalidomide, an immunomodulatory drug, was approved for treatment of relapse/refractory multiple myeloma (RR-MM). In Turkey, we have been used the combination of lenalidomide and dexamethasone (RD) for RR-MM patients after 2010. Therefore, we analyzed efficacy and safety of RD in Turkish patients with RR-MM.

**Aims:** We aimed to evaluate the outcome and the tolerability of the RD in patients with RR-MM who had been treated under the standard clinical practice between October 2010 and June 2016.

**Methods:** This is a retrospective, single center study. Patients' clinical and laboratory data were collected from patient files. The overall and progression free survival (OS and PFS) at 2 years were estimated with the life table method. The univariate analysis (log-rank test) was used to evaluate the variables affecting OS and PFS (univariate analysis). Cox proportional hazards regression was used for multivariate analysis to analyze the independent variables affecting PFS and OS.

**Results:** One-hundred and twenty patients (71 male and 49 female) enrolled in the study. The median age at the start of RD was 64 years (29-84) and the median number of previous line of treatment was 1 (1-4). Seventy-two-two patients (60%) received RD as second-line therapy and 51 of patients (42.5%) treated with autologous stem cell transplantation (ASCT). With regard to the initial dose of lenalidomide, 82 (68.3%) of the patients received the recommended dose of 25 mg per day for 21 days in a cycle of 28 days. Objective response rate (ORR) was observed in 87 patients (72.5%); 23 patients (19.2%) achieved CR. The median follow-up was 14 months (range, 1-72 months), and the median DOR was 19 months (range, 12.4-25.6 months). Median OS and PFS were 32 months (95% CI, 15.8-48.0 months) and 21 months (95% CI, 15.8-26.1 months), respectively. In the multivariate analysis, the independent prognostic factors for OS and PFS were treated with previous ASCT, patients who achieved at least PR, patients receiving RD for more than 12 cycles. Adverse events occurred in 69 of patients (57.5%). Hematological and non-hematological adverse events were found at the same rate (n=47, 39.2%). The treatment discontinuation was due to adverse events in 11.7% (14 patients). The overall incidence rate (IR, events per 100 patient-years) of second primary malignancies (SPMs) was 0.93 (95% CI, 0.04-4.60). The rate of anemia was 12.5% and thrombocytopenia was 9.2% in all grades. Neutropenia (15.8%), fatigue (14.2%) and herpes infections (0.8%) have been reported as most frequent non-hematological side effects.

**Summary/Conclusions:** RD is a safe, well tolerated and effective treatment in patients with RR-MM. Good response, previous ASCT and using more than 12 cycles are associated with better survival. Higher OS and PFS and ORR seem to be related to using RD in the first relapse. Adverse events are manageable and lower with prophylaxis.

**PB1969**

**OPTIMIZING THE MANAGEMENT OF NON-HEMATOLOGICAL ADVERSE EFFECTS RELATED TO LENALIDOMIDE IN RELAPSED MULTIPLE MYELOMA PATIENTS. ONE CENTER EXPERIENCE**

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1Hematology, Hospital Clínico Universitario Virgen de la Arrixaca, El Palmar, Spain

**Background:** During many years, the combination of lenalidomide and dexamethasone (RD) has been an effective treatment for patients with relapsed or refractory Multiple Myeloma (RMM). On the basis of the available evidence, treatment with RD may continue in responding patients until progression or unacceptable toxic effects. The data suggest full dose lenalidomide is important for optimal efficacy and to improve the progression free survival (PFS). Approaches to achieve higher doses of lenalidomide could include continuing therapy in responding patients and proactive adverse effects (AEs) management.

**Aims:** The main aim was to evaluate the incidence of two of most common non-hematologic AEs related to lenalidomide (rash and dystonia) in patients who received RD treatment with RD. The second end points were to evaluate the response of rash after switching the enoxaparin to bemiparin and to evaluate the response of the dystonia after treatment with clonazepam, instead of lenalidomide dose reduction.

**Methods:** We retrospectively reviewed a consecutive cohort of patients with RRM receiving Rd (R: 25 mg on days 1 through 21, d: 40 mg on days 1, 8, 15, and 22) in 28-day cycles until progression or unacceptable adverse effects, from 2011-2016. All patients received thromboprophylaxis with low-molecular-weight-heparin (LMWH) (Enoxaparin 40 mg subcutaneous daily) the first 4 cycles; thereafter, patients were switched to aspirin 100 mg in a day prophylaxis. Bemiparin 7500 anti-Xa IU once-daily dose was employed if enoxaparin was suspended. Clonazepam dose to treat dystonia was 0.5 mg twice daily. Data were analyzed with SPSS statistical v 22.0.

**Results:** Between 2011 and 2016 a total of 65 patients received Rd in our center. Baseline characteristics are shown in Table 1. Patients received a median of 2 previous regimens (range 1-8). 51.5% of the patients had undergone one previous autologous stem-cell transplant (ASCT). Rash occurring in 12.3% of patients (grade 2), all of them were concurrently receiving enoxaparin. All rashes resolved switching the enoxaparin to bemiparin, maintaining same dose of lenalidomide. Neither treatment with esteroids or antihistaminic were administrated. Dystonias were responded in 23.1% of patients (grade 2), all of them dissapeared after treatment with clonazepam without lenalidomide dose reduction.

**Table 1.**

**Summary/Conclusions:** Rash and dystonias are frequent adverse effects of immunomodulatory drugs (IMiDs), particularly lenalidomide, often leading to treatment discontinuation and decreasing the potential benefits to patients. According to our data, the rash could be due to synergism between enoxaparin and lenalidomide. In most cases, switch LMWH letting not to reduce lenalidomide dose in order to optimize the benefit of the treatment. Clonazepam, a benzodiazepine, is useful to treat dystonias related to lenalidomide.

**PB1970**

**PROLONGED THROMBOPROPHYLAXIS IN PATIENTS TREATED WITH LENALIDOMIDE AND DEXAMETHASONE DOES NOT SEEM STRICTLY MANDATORY TO PREVENT LATE THROMBOTIC EVENTS**

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**Background:** Risk of venous thromboembolism (VTE) in general population is 1% annually, significantly higher in oncologic setting, in particular with Multiple Myeloma (MM). Treatment with Lenalidomide plus Dexamethasone represents an additional risk factor for VTE, with most of VTE events observed in the first six months since therapy starting. No definitive data are available on the more appropriate duration of thromboprophylaxis (TP) in patients treated with lenalidomide.

**Aims:** To explore: I) the incidence of late thrombotic events in a real world population of relapsed MM, addressed to Lenalidomide plus low dose Dexamethasone treatment (Len-dex), and concomitant TP with low molecular weight heparin (LMWH) performed for the first 4-6 months of therapy, without TP maintenance, II) the possible correlation between the presence of thrombotic risk factors and the occurrence of a late VTE.

**Methods:** We performed a retrospective analysis, after regular approval of local ethic committee, on chart data of 103 patients (pts) with relapsed MM treated with Len-dex according to label indication between January 2003 and December 2016 at our single centre institution. VTE prophylaxis was performed with daily dose of subcutaneous LMWH 4000 IU for 4-6 months, with no further TP, regardless of the presence of thrombotic risk factors.

**Results:** Main features of patients on study were: median age 66.3 years (range 41.9-85.2 years), median previous line of therapy 3 (range 1-7), time from diagnosis to lenalidomide starting 33.3 months (range 0.3-159.9 months), median duration of Lenalidomide treatment 8 months (range 0.4-65.2 months) with the following response: sPR 96%, CR 7%. Table 1 shows type and distribution of risk factors for VTE. In details median number of VTE risk factors per patient was 2 (range 0-6), 58.2% of pts had ≥2 risk factors, 41.8% of pts had 0-1 risk factor for VTE. Median duration of TP is 4.8 months (range
0.4-6 months). No hemorrhagic events were observed during LMWH. Cumulative incidence of VTE was 11.7% (12/103 pts), similar to that previously reported in the literature in patients with continuous TP. The median time from relapse to starting LMWH was 1.7 years (range 0.1-9.2 years).}

**Table 1. Baseline distribution of risk factors for thrombosis in the population on study.**

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1-44 years</td>
<td>57%</td>
</tr>
<tr>
<td>45-64 years</td>
<td>42%</td>
<td></td>
</tr>
<tr>
<td>≥65 years</td>
<td>11%</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>53%</td>
</tr>
<tr>
<td>Female</td>
<td>47%</td>
<td></td>
</tr>
<tr>
<td>ISS stage</td>
<td>ISS1</td>
<td>19%</td>
</tr>
<tr>
<td>ISS2</td>
<td>42%</td>
<td></td>
</tr>
<tr>
<td>ISS3</td>
<td>60%</td>
<td></td>
</tr>
<tr>
<td>VD</td>
<td>21%</td>
<td></td>
</tr>
<tr>
<td>VCD</td>
<td>6%</td>
<td></td>
</tr>
<tr>
<td>VPI</td>
<td>35%</td>
<td></td>
</tr>
<tr>
<td>VPII</td>
<td>21%</td>
<td></td>
</tr>
<tr>
<td>VPIII</td>
<td>12%</td>
<td></td>
</tr>
<tr>
<td>VPIIV</td>
<td>13%</td>
<td></td>
</tr>
<tr>
<td>LDH</td>
<td>3.4 g/dL (range 1.0-5.3)</td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>10.0 mg/dL (range 8.7-20.2)</td>
<td></td>
</tr>
<tr>
<td>GFR</td>
<td>49.3 mL/min (range 3.6-114.2)</td>
<td></td>
</tr>
<tr>
<td>IgG type</td>
<td>IgG</td>
<td>60%</td>
</tr>
<tr>
<td>IgA</td>
<td>32%</td>
<td></td>
</tr>
<tr>
<td>IgD</td>
<td>1%</td>
<td></td>
</tr>
<tr>
<td>Bence-Jones protein</td>
<td>30%</td>
<td></td>
</tr>
<tr>
<td>Non-secretory protein</td>
<td>2%</td>
<td></td>
</tr>
</tbody>
</table>

**Summary/Conclusions:** This study shows that LMWH is effective and well tolerated for early VTE prophylaxis during Lenalidomide plus low dose Dexamethasone. Incidence of late VTE without TP maintenance is similar to that reported with long-term antplatelet therapy. We found no difference in factors predisposing for thrombosis among patients developing or not VTE, with a not negligible proportion of concomitant adverse events observed nearby VTE occurrence.

**PB1971**

**ELDERLY PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA IN THE REAL WORLD**

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**Background:** Many new agents for multiple myeloma (MM) were launched during the last decade, and the clinical trial using such new agents showed promising results for MM patients. However, clinical course of elderly patients with newly diagnosed MM (NDMM) in the real world is different from the results of clinical trial.

**Aims:** We examined the clinical parameter to assess survival in elderly patients with NDMM in clinical practice.

**Methods:** We performed a retrospective study involving 125 elderly NDMM patients from April 2012 to September 2015. Patients aged 60 years or older, who were ineligible for autologous stem cell transplantation, were selected. The study included 57 males and 68 females, with median age at diagnosis of 74 years (range: 60-95 years). ECOG performance status at diagnosis were 0-1, 47%; 2-4, 21%; 5-10, 32%. The median time from relapse to starting LMWH was 1.7 years (range 0.1-9.2 years).

**Results:** Of 125 patients, 76 patients received bortezomib based therapy (VMP, 48; VD, 21; VCD, 6), 6 patients received lenalidomide based therapy (Ld, 6), 10 patients were received MP therapy, 19 patients received dexamethasone therapy (high dose, 16; low dose, 3), 1 patient received radiation therapy as first line therapy, and 13 patients received only supportive care due to their fragility. After induction therapy, the overall response rate (at least partial response, PR) was 52.7% (stringent complete response (sCR) 0.3%, CR 4.5%, very good PR 16.1%, PR 29.5%). Overall survival (OS) was 74.5% at 1 year, 66.2% at 2 years with median follow-up of 19 months (range 1-52) for patients who were still alive at the date of last contact and 14 months (range 1-52) for entire cohort. Death occurred in 41 patients during the follow-up period. International staging system (ISS), with ISS1, 19; ISS2, 42; ISS3, 60; N/A, 4, can divide elderly patients into three distinct survival groups (P <0.001) (Figure 1A). Univariate and multivariate analysis showed a lower OS was associated with eGFR lower than 40 ml/min (HR 2.279, 95%CI 1.152-4.510) (Figure 1B) and albumin level (mean 3.4 g/dL [range 1.0-5.3]).

**Summary/Conclusions:** Survival of patients treated with bortezomib or lenalidomide as an induction therapy was better, while not statistically significant (P=0.066) than those who were not.

**PB1972**

**RETROSPECTIVE ANALYSIS OF 121 MULTIPLE MYELOMA PATIENTS USING THE R-ISS PROGNOSTIC STAGING SYSTEM AND RESPONSE TO FIRST LINE OF TREATMENT**

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1Hematology, Kuwait cancer control centre, Kuwait, Kuwait

**Background:** The International Myeloma Working Group has developed the R-ISS (Revised International Staging System) as a simple and powerful prognostic staging system. We collected the LDH level and the cytogenetics of a group of patients and studied the difference between the ISS (International Staging System) and the R-ISS (Revised International Staging System) for those patients.

**Aims:** To evaluate and compare between the ISS and the R-ISS for a group of patients treated in Kuwait Cancer Control Centre.

**Methods:** A retrospective analysis of the data collected from 121 patients registered as multiple myeloma from 2011-2015. Of the patients presented to our centre after initial diagnosis and starting the first line treatment, the patients were categorised according to age, gender, ISS stage, R-ISS stage, first line therapy and response.

**Results:** We recognised increase of the number of the yearly diagnosed patients with myeloma 2.48% of patients the actual date of diagnosis was before 2011 but only 1.75% of patients the actual date of diagnosis was before 2012 which is 1.75% lower than the actual date of diagnosis. The patients were categorised into 14 groups according to age, gender, ISS stage, R-ISS stage, first line therapy and response.

**Background:** The International Myeloma Working Group has developed the R-ISS (Revised International Staging System) as a simple and powerful prognostic staging system. We collected the LDH level and the cytogenetics of a group of patients and studied the difference between the ISS (International Staging System) and the R-ISS (Revised International Staging System) for those patients.

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**Summary/Conclusions:** Survival of patients treated with bortezomib or lenalidomide as an induction therapy was better, while not statistically significant (P=0.066) than those who were not.
even both. First line treatment 55% of the patients received Bortezomib based triple therapy 22%, lenalidomide and 4 CTD (Cyclophosphamide, Thalidomide, Melphalan), 7% RD (Lenalidomide, Dexamethasone), 3% CyBord (Cyclophosphamide, Bortezomib, Dexamethasone), 3%RV (Lenalidomide, Bortezomib), 2% Thal-Dex (Thalidomide, Dexamethasone), 2% RT (local Radiotherapy), 2% WatchfulWait, 1% MP (Melphalan, Prednisone) and 3% refused for treatment and lost follow up.

Table 1.

<table>
<thead>
<tr>
<th>ISS stage</th>
<th>% of patients</th>
<th>ISS stage</th>
<th>% of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>31%</td>
<td>Stage II</td>
<td>26%</td>
</tr>
<tr>
<td>Stage III</td>
<td>47%</td>
<td>Stage III</td>
<td>56%</td>
</tr>
<tr>
<td>Plasma-cell myeloma</td>
<td>2%</td>
<td>Plasma-cell myeloma</td>
<td>2%</td>
</tr>
<tr>
<td>Uteacon</td>
<td>4%</td>
<td>Uteacon</td>
<td>4%</td>
</tr>
</tbody>
</table>

Summary/Conclusions: Applying the RISS system to myeloma patients is a very effective and easy method to categorise myeloma patients, a significant number of patients in Kuwait are diagnosed as stage III, with median age of 56 years although the use of novel therapies shows excellent response to most of them.

PB1973
FEASIBILITY/PHASE II STUDY OF MYEOLABLATIVE BEAM ALLOGENIC TRANSPLANTATION FOLLOWED BY ORAL IXAZOMIB MAINTENANCE THERAPY IN PATIENTS WITH HIGH RISK MYELOMA

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Background: While the role of allo-HCT in MM remains controversial several studies have shown encouraging PFS and OS with this treatment even in patients with high-risk myeloma (HRM). HRM manifests with early relapses and refractoriness. Median OS is 2.5 years despite aggressive therapy with novel agents. Post auto-HCT maintenance with lenalidomide is considered standard of care, but post allo-HCT maintenance presents unique challenges and has not been well studied. Ixazomib (Ixa) is a new oral proteasome inhibitor with activity in MM refractory resistant patients, and is a promising agent in the maintenance setting.

Aims: Here we present preliminary results for this trial. The primary objective is safety defined as day 100 transplant related mortality (TRM), and safety of Ixa maintenance (incidence of grade III-IV GvHD and Ixa related toxicity). Other objectives include determination of efficacy (ORR, PFS, MRD for CR), the ability to start Ixa, and quality of life.

Methods: The protocol was approved by a local institutional review board and ethics committee. The study was conducted in accordance with the Declaration of Helsinki. All subjects provided written informed consent prior to treatment. Eligible subjects were those 18-75 years old newly diagnosed with myeloma treated with autologous HCT or allogeneic transplantation. The BEAM conditioning regimen includes: BCNU 200 mg/m2 on day -7; cytarabine 400 mg/m2 daily day -5 to day -2; etoposide 200 mg/m2 daily day -5 to day -2; melphalan 140 mg/m2 on day -1. Oral Ixa 4mg on days 1 and 14 of a 28-day cycle. Ixa may start between day 90 and 180 post HCT, and continue for up to 24 cycles.

Results: Six subjects were enrolled, 3 at OHSU and 3 at Duke, from Sept 2015 to Dec 2016. Median age of 51 (range 46-57), 2 female, and all of white race. High risk factors: del(17p), t(14;16), t(14;16), t(14;20), amp1q gain or del1p, del13q by conventional karyotyping, hypodiploidy, high-risk GE, B2M >5.5mg/l, plasmablastic morphology (>2%), or relapsed plasma cell leukemia; 6/8 HLA matched unrelated (MUD) or sibling donor. The BEAM conditioning regimen includes: BCNU 200 mg/m2 on day -7; cytarabine 400 mg/m2 daily day -5 to day -2; etoposide 200 mg/m2 daily day -5 to day -2; melphalan 140 mg/m2 on day -1. Oral Ixa 4mg on days 1 and 14 of a 28-day cycle. Ixa may start between day 90 and 180 post HCT, and continue for up to 24 cycles.

Summary/Conclusions: Although this is very early data, it is the first clinical trial to report the use of BEAM conditioning followed by Ixa maintenance for relapsed HRM. Thus far stopping rules have not been met, with expected toxicities occurring.

PB1974
EPIDEMIOLOGY OF MULTIPLE MYELOMA. THE GRANADA MYELOMA REGISTRY

R. Rio Tamayo1, D. Sánchez-Rodríguez2, J. Sainz Pérez3, J. Jiménez Moleón3, M. Pérez Sánchez3, M. Jurado Chacón1
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Background: The Granada Myeloma Registry is the second largest single institution population-based registry (Rios-Tamayo et al, 2015) of multiple myeloma (MM) referenced to date. Here we update and point out the epidemiological variables of interest.

Aims: To highlight the importance of the epidemiological perspective in the knowledge and outcome of MM.

Methods: From January 1985 to February 2017 all consecutive patients diagnosed with MM at our institution have been registered, including clinical, biological and socio-demographic variables, as previously reported. A comprehensive approach to comorbidity was recorded as well as diagnostic and treatment delay. Overall survival (OS) was estimated by the Kaplan-Meier method.

Results: 700 patients have been included in the registry, 343 men (49%) and 357 women. All cases have their place of residence in the Granada province. The median age was 67 years (range: 9-123). The race was Caucasian in 99.8%. In relation to occupation, 18.4% were skilled or elementary agricultural workers. Only 8% had a previously documented precursor disease (solitary plasmacytoma, monoclonal gammopathy of undetermined significance, or smoldering MM), and 14 patients (2%) remain alive with smoldering MM without progression. The subtype of MM is IgG 55.6%, IgA 24.8%, Light chain only 15.9%, Non-secretory 3%, IgD 0.8% and IgM 2.0%. The International Staging System is known in 378 patients (25.9%), 23 patients (1.6%) and 3 (4.8%). Baseline performance status (ECOG) was: 0 (4.7%), 1 (41.1%), 2 (26.7%), 3 (21.7%), and 4 (5.9%). Comorbidity was assessed in 498 patients. 30.6% of patients were obese at the moment of diagnosis. 8.2% had other previously known or synchronous neoplasms. 150 patients (30.1%) had three or more comorbidities. The median diagnostic delay was 4.1 months (0.1-80) and median treatment delay was 13 days. 44 patients (6.3%) were very unfit and they did not receive active treatment. Information about stem cell transplant is available in 606 cases: 151 of them (24.9%) received a first autologous transplant. Median OS for the whole cohort was 43.1 and 22.4 months for patients younger than 65 years or 65 years or older, respectively (p<0.001). For patients younger than 65 years or older, median OS is not reached for younger than 65 and 40.4 months for the elderly (p=0.001). Information about the main cause of death is available in 230 patients: 101 (43.9%) of them died by infection.

Summary/Conclusions: MM is a very heterogeneous disease from a clinical, biological and epidemiological perspective. The distribution by sex is identical. Farmer is the most frequent occupation. Almost one in three patients are obese, and one in ten had another prior or associated neoplasia. Infection is the leading cause of death. Information derived from population-based registries may help to complement data from clinical trials.

PB1975
REAL WORLD USE OF IXAZOMIB WITH LENALIDOMIDE AND DEXAMETHASONE FOR PATIENTS WITH RELAPSED AND RELAPSED REFRACTORY MULTIPLE MYELOMA

M. Ziff1, S. Cheesman1, C. Kynaku1, A. Mehta1, X. Papanikolau1, N. Rabini1, A. Wechalekar1, K. Yong1, R. Popat1
1University College London Hospitals NHS Foundation Trust, London, United Kingdom

Background: Ixazomib (Ixa) is a novel oral proteasome inhibitor (PI) approved in combination with lenalidomide and dexamethasone (IRD) for the treatment of relapsed/refractory multiple myeloma (MM). This was based on the TOUR-MALINE-MM1 trial which demonstrated a progression free survival benefit over RD. However real world use often differs to clinical trials due to heterogeneous patient selection, more flexibility with dosing intensity and country specific prescribing practices/funding restrictions.

Aims: To characterise real world use of IRD by demographics, response rate (RR) and progression free survival.

Methods: This was a retrospective review of patients sequentially treated with IRD at a large UK Haematology Centre. Patients received Ixa 4mg D1, 8, 15 with lenalidomide (dose as per label) days 1-21 and dexamethasone 40mg weekly or as tolerated every 28 days until disease progression or intolerance. In some cases, Ixa was added later to RD. RR and PFS were assessed according to IMWG criteria and haematological toxicities graded by CTCAE 4.0 criteria.

Results: Up to 314th October 2018, 30 patients were treated with the IRD schedule. Median age was 65 years (32-75), male (57%), ISS: stage I 18 (60%), stage II 4 (13%), stage III 8 (27%). 7 patients had a median of 2 (2-5) prior lines of therapy. All patients had previous treatment with a proteasome inhibitor (PI) (29 bortezomib, 5 carfilzomib) and 8 (27%) were refractory to a PI. 3 (10%) had prior lenalidomide and all remained sensitive. 23 (77%) had a prior autologous transplantation.
with successful salvage, contributing to the observed long duration of local therapy compared to national averages. Pomalidomide was highly effective at rescuing patients failing lenalidomide-based regimes and well tolerated.

**PB1977**

**APPLICATION OF CONDITIONING REGIMEN WITH BUSULFAN AND CYCLOPHOSPHAMIDE IN AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA**

Y. Xu1,2, C. Fu1,2,*, Y. Yao2, W. Yao2, S. Jin2, L. Yan2, J. Shang2, X. Zhu2, A. Sun2, D. Wu1,2 1Institute of Blood and Marrow Transplantation, Collaborative Innovation Center of Hematology, Soochow University, 2The First Affiliated Hospital of Soochow University, Jiangsu Institute of Hematology, Suzhou, China

**Background:** Busulfan is the most commonly used drug in conditioning regimens for hematopoietic stem cell transplantation: and high-dose melphalan (MEL) is the standard conditioning regimen in autologous stem cell transplantation (ASCT) for multiple myeloma (MM).

**Aims:** Evaluate the safety and efficacy of BUCY (busulfan and cyclophosphamide)-conditioning regimen for autologous hematopoietic stem cell transplantation (ASCT) in patients with multiple myeloma (MM).

**Methods:** We retrospectively analyzed the clinical data of 72 MM patients who received transplantation in the Hematology Department of the First People’s Hospital of Soochow University from May 2012 to June 2015. Among them, 36 patients underwent BUCY regimen while the others received high dose melphalan. Those were compared between the two groups including the complications of hematopoietic reconstitution and the post-transplantation efficacy.

**Results:** There were no significant differences in age, stage, induction therapy, mobilization method between the two groups. The transplant-related adverse events were similar in both groups but the incidence of pulmonary infections was lower in the BUCY group. The incidence of bloodstream infection were slightly higher in the BUCY group. The median time to neutrophil engraftment in the BUCY and HDM groups were 10(8-17) days versus 10(9-13) days, taking the same time on average (P=0.046). On the other hand, the median time to platelet engraftment was 10(8-18) versus 11(9-47) days accordingly (P=0.017). The TRM in both group was 2.7%. The SGR/CR rates after ASCT (47.2% and 50.0%) were higher than those before it (38.9% and 26.6%), in both groups. In the BUCY group, the median follow-up was 12.5 (0-26) months. Six patients (16.7%) underwent disease progression. The 2-year progression-free survival (PFS) rate was 68%. Correspondingly, in the HDM group, the median follow-up time was 23 (0-38) months. Fifteen patients (41.7%) developed disease progression and the 2-year PFS rate was 55%.

**Summary/Conclusions:** The BUCY regimen is a safe and effective therapy for ASCT in patients with multiple myeloma. Besides, BUCY regimen is not inferior to HDM regimen. In conclusion, BUCY regimen may replace HDM regimen as a standard conditioning regimen for ASCT in multiple myeloma.

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**PB1976**

**EFFICACY AND TOLERABILITY OF LENALIDOMIDE AND POMALIDOMIDE IN RELAPSED/REFRACTORY MYELOMA PATIENTS IN A REAL WORLD STUDY**

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**Background:** New agents have revolutionised the treatment of multiple myeloma. Immunomodulatory drugs (IMiD) such as lenalidomide and pomalidomide are approved as second line drugs leading to improved progression free survival (PFS) and overall survival (OS). Published studies include exclusion criteria such as cytopenias, renal dysfunction and poor performance status (common in multi-relapsed patients), raising the question regarding the benefit of IMiD therapy in the real-world setting.

**Aims:** In our study we aimed to describe the real-world experience of the use of lenalidomide followed by pomalidomide rescue in a relatively elderly co-morbid cohort over a 4 year period and compare this to national averages. We reviewed IMiD efficacy, including sequential lenalidomide followed by pomalidomide, together with tolerance.

**Methods:** Records of delivered chemotherapy cycles were retrieved from local pharmacy data and national averages from Celgene ePAF data. Outcome data collected from clinical notes and laboratory results.

**Results:** We collected data on 46 patients treated between 2011-2014 with lenalidomide, 17 whom progressed to receive pomalidomide. The median age at initial diagnosis was 71 years, with median age at starting lenalidomide 77 years (range 36-94). This gave an average of 5 years from diagnosis to commencing lenalidomide (range 1-15 years). Myeloma subtypes included IgG 28/46, IgA 11/46, light chain disease 4/46 and 3 with IgD and non-secretory myeloma. High risk cytogenetics [17p-, (4;16), t(4;20), hypodiploidy, chromosome 1 abnormalities] were identified in 9/46 and 16/46 were high-risk based on biomarker staging (ISS). All patients had at least 1 preceding line of therapy before starting lenalidomide, average 2 lines (range 1-6). Prior treatment included alkylating agents/steroid doublets, thalidomide combinations, bortezomib-based therapy and autograft. National average for the% of patients reaching cycle 26 was 16% compared to our local cohort of 31%. This included patients receiving lenalidomide for severe renal impairment or cytopenias. In the patient group between 65-75 years of age, 50% reached cycle 26 compared to the national average of 16%. Average duration on treatment was 15 months. (Local-cohort). Lenalidomide-treatment breaks occurred in 16 patients with a median of 5 months (infection, cytopenias, live events, patient choice, etc.). Nine patients had at least 1 prior ASCT, median number of treatment cycles in those who progressed to pomalidomide was 12.8 (n=17), which is double that of the national average reported in seminal trials. These patients had few treatment breaks and treatment was well tolerated (pomalidomide doublets or triplets).

**Summary/Conclusions:** We conclude from this real-world retrospective review of 2nd and 3rd line IMiD therapy that these salvage regimes are highly effective. Patients on lenalidomide monotherapy post triplet/duplet induction were often re-escalated back onto dexamethasone and alkylator (IV/oral) based regimes.

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**PB1978**

**MULTIPLE MYELOMA WITH CENTRAL NERVOUS SYSTEM INVOLVEMENT, 12 CASES AND REVIEW OF THE LITERATURE**

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**Aims:** To review a series of 12 cases of MM and CNS involvement. A comprehensive review of the literature will be performed to review the incidence, diagnosis, management and outcomes of MM with CNS involvement.

**Methods:** Clinical data from 12 patients with MM and CNS involvement were collected. Their medical records were reviewed and the relevant literature were searched.

**Results:** Between 2008 and 2015, 12 cases of MM with CNS involvement were identified. The median age was 55 years (range, 40-75 years). The most common presenting symptom was bone pain (7/12). Initial cranial magnetic resonance imaging (MRI) showed abnormal findings in 11/12 patients. The most frequent abnormalities seen were low signal intensity on T2-weighted images and enhancement. The most common CNS sites involved were the brainstem (9/12), cerebellum (7/12), and spinal cord (5/12). The median time from the diagnosis of MM to CNS involvement was 21 months (range, 1-127 months). The median follow-up time from the diagnosis of CNS involvement was 36 months (range, 1-108 months). Treatment did not improve survival. The median survival from the diagnosis of CNS involvement was 23 months (range, 1-60 months).

**Conclusion:** MM with CNS involvement is a rare event but can have a significant impact on survival. Early diagnosis and identification of CNS involvement can improve patient management and overall survival.
progression was 23.9 (3-65) months. Eight patients presented with cerebral nerve palsies, 2 with paraplegia, 1 with hemiplegia and 1 with headache. CSF cytospin or flow cytometry was positive in 7, MRI or CT supported the diagnosis in 4 patients. Treatment consisted of combination chemotherapy, intrathelial chemotherapy, cranio-caudal radiotherapy and imids with various success. The PFS and OS from CNS progression was 63 and 125 days. Two patients survived for over a year (427 and 778 days), both responded in terms of CNS symptoms to imid-based combination therapy and one had cranio-caudal radiotherapy (Figure 1).

Figure 1.

Summary/Conclusions: CNS progression in MM has a particularly poor prognosis as it represents a late stage of an aggressive relapse which often shows chemo-refractoriness. The differential diagnosis includes infection, autoimmune or vascular disease of the CNS as well as paraneoplasia and drug toxicity. The CNS penetration of the effective myeloma drugs is poor except for the imids, and drugs with CNS availability are usually not very effective in refractory MM.

PB1979

DARATUMUMAB: CHALLENGES OF INTEGRATING THIS NEW THERAPY INTO STANDARD CARE

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Background: Daratumumab (Darzalex) is the first anti-CD38 human Monoclonal Antibody approved for Multiple Myeloma (MM). Targeting the CD38 antigen on the surface of MM cells it causes apoptosis, and has an immune modulatory tumour lysis effect. Success in Clinical trials meant that this drug, administered as single agent, or in combination with other novel therapies (Lenalidomide or Bortezomib), received accelerated FDA Approval in the US. It is now being introduced into standard hospital care.

Aims: Daratumumab presents unique challenges to the delivery of risk management, due to effects on some blood and bone marrow testing, and to the Infusion Related Reactions (IRRs) seen at the outset of treatment. This poster will highlight important aspects of the treatment pathway for this new therapy, from a single centre perspective.

Methods: We outline the pathways integrated at MDT level; patient characteristics and adverse event profiles of the 15 myeloma patients we have treated with Daratumumab, in a standard service setting.

Results: Daratumumab affects certain laboratory tests so samples should be clearly identified. Relevant laboratory teams need to be aware of the methods used to process samples. Daratumumab binds to CD38 on Red Blood Cells, and therefore with Cross Match Compatibility testing and Antibody Screening. Obtaining RBC Products for patients receiving Darar will take longer, requiring up to 48 hours’ notice. Cross match samples taken prior to treatment provide the National Blood Service Laboratory with a baseline antigen profile to aid selection of suitable blood products. Dara is detected during Paraprotein Electrophoresis; our lab use a Sebia capillarys 2 analyser to separate the Dara band for accurate quantification of suitable blood products. Dara is detected during Paraprotein Electrophoresis; our lab use a Sebia capillarys 2 analyser to separate the Dara band for accurate quantification.

Summary/Conclusions: Education, to include Blood Transfusion, Protein and Histopathology laboratory, and High Dependency Unit staff, in the key aspects of monitoring and risk management are an important part of integrating this new therapy to the treatment pathway for myeloma patients. Daratumumab is likely to become an important treatment for improving both Outcomes and Quality of Life for Myeloma patients going forward.

PB1980

MULTIPLE MYELOMA IN HIV+ PATIENTS LITERATURE REVIEW AND OWN CASE

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Background: Multiple myeloma (MM) and HIV infection in AIDS stage until now its considered not to be associated. Recently new ideas appear in the literature such as influence of HAART on the treatment outcomes of MM in HIV negative patients.

Aims: To find literature sources on multiple myeloma in HIV positive patients and elucidate the problem of this association. evaluate the impact of HAART in multiple myeloma.

Methods: Patients were retrospectively identified out of 39 cases of MM and HIV from Pubmed/Medline from 1983 to 2017, and own case reported.

Results: Patients with MM and HIV infection did not differ significantly from the MM in HIV-negative with respect to age, gender, stages and renal function. Effects of HAART on levels of serum M-protein HAART itself has been reported to decrease M-protein in an HIV+ patient with MM. We determined whether HAART alone, in the absence of MM treatment, had any effects on the levels of serum M-protein in HIV+MM patients. Depending on the interval between the discovery of the HIV infection HAART treatment initiation, and the diagnosis of MM and initiation of its treatment. The overall and progresion free survival of HIV+MM patients on HAART was superior to that of non-HIV-MM patients reported in the literature. The majority of HIV+ MM patients who had long-term follow-up in our study did not show clinical symptoms of MM and were free of serum-M protein after primary MM therapy in the presence or absence of HAART and maintained treatment with HAART alone. Although MM is not an AIDS-defining illness, meta-analyses of large population studies reveal an increased risk of MM in HIV/AIDS patients. HIV infection is commonly associated with B cell hyperproliferation, as indicated by polyclonal hyperglobulinemia and the development of various autoantibodies. This is presumed to be usually due to these CD4 deficient patients’ inability to control Epstein-Barr virus infections, which immortalize B cells. This may help to explain the increased incidence of MM in HIV+ patients. However, HIV can neither infect B lymphocytes or plasma cells, nor drive their malignant transformation. Some authors are going to treat multiple myeloma in HIV seronegative patients with HAART in combination with chemotherapy (Geling Lia and co-authors, Leukemia Research, 2014). A 38 year-old Russian male presented at the Moscow clinical Center in 2015 with pronounced ossalgya and inability to move. Total protein 135 g/l with 81.7 g/l of IgG-k M-protein and no presence of Bence Jones protein. Bone skeletal survey showed multiple generalized lytic lesions. Bone marrow aspirate and biopsy showed 46% plasma cells. Serum creatinine ~ 104 mkmoll. HIV and hepatitis C (genotype 1a) screening test were positive, confirmed with Western blot analysis. The CD4 count was 290 cells, HIV viral load 1500 copies/ml, hepatitis C viral load 14.2 mln copies. He was started on HAART, combined with chemotherapy 5 courses of CP+V+PM+ and 7 V-PM. In 2017 total serum protein~ 97.3 g/l, M-protein 31.2 g/l, serum creatinine 63.0 mkmoll. Now he is active without any bone pain receives Pegasys and lamivudine (Table 1).

Summary/Conclusions: Patients with MM and HIV infection did not differ significantly from the MM in HIV-negative with respect to age, gender, stages and renal function, and treatment with addition of HAART.Recently was reported that HAART itself may reduce and even remove m-gradient in HIV positive
patients. It is considered to include HAART in HIV negative patients with MM. The problem of MM and HIV/AIDS association remains unclear and needs to be elucidated.

Table 1.

<table>
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<tr>
<th>Age</th>
<th>Characteristic of patients with HIV infection and MM</th>
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<tr>
<td>M-Gradient</td>
<td>Age (mean ± SD)</td>
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<tr>
<td>G2a</td>
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PB1981

OPTIMIZATION OF APPROACHES FOR STEM CELL MOBILIZATION FOR AUTOLOGOUS STEM CELL TRANSPLANT FOR MULTIPLE MYELOMA: PRACTICAL CONSIDERATIONS

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Background: Autologous stem cell transplant (ASCT) is a well-established treatment for myeloma. However, the optimal strategy for stem cell mobilization remains undefined. The goal of mobilization is to collect adequate stem cells for at least 2 ASCT (4x10^6/kg), with the minimum apheresis sessions and toxicities such as febrile neutropenia.

Aims: We aim to compare stem cell mobilization using granulocyte colony stem cell factor (GCSF) only (steady state), high dose cyclophosphamide (4 g/m2) with GCSF or low dose cyclophosphamide (2 g/m2) with GCSF.

Methods: We performed a retrospective analysis of 79 patients mobilized with GCSF only from mid-2014 to Aug 2016 with 32 patients mobilized using high dose cyclophosphamide and 23 patients with low dose cyclophosphamide during a similar period.

Results: Patients undergoing steady state collection required a median of 2 days for adequate collection, in comparison to 1 day for both high and low dose cyclophosphamide. Addition of plerixafor was required in 27% of patients on steady state collection, in contrast to 3.1% and 15% of patients on high and low dose cyclophosphamide respectively. The mean yield of CD34+ x 10^6/kg cells collected was 5.39, 9.14 and 8.5 for steady state, high, and low dose.

There was no significant difference in time to engraftment despite a lower dose of CD34+ cells reinfused for the steady state cohort. Admission for febrile episodes was observed that 60.7% patients mobilized with high dose cyclophosphamide, as compared to 13% of patients on the lower dose regime and none in the steady state cohort. Patients mobilized with cyclophosphamide had a longer interval between stem cell collection and transplant (median of 20, 42 and 34 days respectively for steady state, high dose and low dose). However, we observed that 60.7% patients with steady state mobilization had increases in their myeloma markers during this period, in contrast to biochemical improvement in 50% of patients mobilized with high dose cyclophosphamide and 26% with low dose cyclophosphamide.

Summary/Conclusions: All 3 strategies for stem cell mobilization have their practical considerations. Currently, we can conclude that MFC could be considered as the method of choice for MRD monitoring in MM. If the disease is measured, then, indeed, enough to evaluate only the M-gradient level of serum. The study included 51 patients MM, average age - 54 years (36-70 years), who underwent assessment of MRD from November 2014 to February 2017. According to the classification Durie-Salmon the vast majority of patients (n=40) had III stage of disease, 8 patients – II and 2 patients – I. Response to treatment was assessed according to standard EBMT criteria. At the time of MRD assessment 20 patients were in CR, 8 had a partial response (PR) and 15 had a resistant disease; 5 patients had a primary MM, 3 patients were in the progression phase. The goal of the study was to compare high-dose chemotherapy with autologous SCT (n=42). Re-evaluation of MRD after therapy was managed to hold in 36 patients at a mean of 3.1 months (1.9-5.7, min-max).

Analysis was performed using a FACSScantoll flow cytometer (BD) and FACSDiva software (BD). Instrument performance was checked daily by recording fluorescence intensity with calibrating beads (Cytometer Setup and Tracking from BD Biosciences). Whole BM was estimated using combination of surface and intracellular staining CD38/CD56/CD138/cytKappa. The sensitivity of our panel MRD is 0.01% (i.e. 10^-4).

Results: Among patients in CR (n=20) confirmed the absence of MRD in 6 patients, but 14 CR patients were MRD positive. MRD was detected in all patients with PR and resistant disease (n=31). The relative content of abnormal plasma cells in CR patients with MRD positive (n=14) was significantly lower than that in PR/resistant patients (n=31): 0.085 (0.026-0.271%) versus 1.3% (0.203-5.9%), pU=0.000092. PR patients (n=8) had a lower relative content of abnormal plasma cells (as expressed tendency), than patients with resistant disease (n=15): 0.286% (0.177-1.129%) versus 1.48% (0.9-0.8%), pU=0.053. Besides the relative content of abnormal plasma cells in PR/resistant patients (n=31) correlated with the serum M-gradient concentration (r=0.42, p=0.019) and the lower dose of GCSF (r=0.54, p=0.0017).

Summary/Conclusions: Currently, we can conclude that MFC could be considered as the method of choice for MRD monitoring in MM. If the disease is measured, then, indeed, enough to evaluate only the M-gradient level of serum. If the M-gradient is not defined, it is necessary to assess the number of abnormal plasma cells in the BM and strive for the high-quality responses at the time of transplantation. And also it can help us to regulate duration of maintenance therapy.

PB1982

MINIMAL RESIDUAL DISEASE MONITORING IN MULTIPLE MYELOMA PATIENTS BY FLOW CYTOMETRY: A SINGLE CENTER EXPERIENCE

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Background: Multiple myeloma (MM) is a malignant disease characterized by an increased number of clonal (abnormal) plasma cells in the bone marrow (BM). High-dose chemotherapy followed by autologous peripheral blood stem cell transplantation (SCT) is used for the treatment of young MM patients and produces a high rate of complete remissions (CR). Recent trials with novel agent combinations alone have also resulted in high CR rates, even among old patients, high-risk patients and relapse/refractory MM. Unfortunately, most patients have a recurrences of the disease. This is due to the persistence of residual tumor cells, known as minimal residual disease (MRD), responsible for tumor relapse.

Aims: BM samples from 51 MM patients who had achieved partial or complete response or were resistant after chemotherapy, including autologous SCT, were evaluated by multiparameter flow cytometry (MFC). The study was conducted to assess the quality of remission, the correlation between the number of abnormal cells of BM and other signs of disease activity, readiness of patients for autologous SCT.

Methods: The study included 51 patients MM, average age - 54 years (36-70 years), who underwent assessment of MRD from November 2014 to February 2017. According to the classification Durie-Salmon the vast majority of patients (n=40) had III stage of disease, 8 patients – II and 2 patients – I. Response to treatment was assessed according to standard EBMT criteria. At the time of MRD assessment 20 patients were in CR, 8 had a partial response (PR) and 15 had a resistant disease; 5 patients had a primary MM, 3 patients were in the progression phase. Most of the patients were under treatment with high-dose chemotherapy with autologous SCT (n=42). Re-evaluation of MRD after therapy was managed to hold in 36 patients at a mean of 3.1 months (1.9-5.7, min-max).

Analysis was performed using a FACSScantoll flow cytometer (BD) and FACSDiva software (BD). Instrument performance was checked daily by recording fluorescence intensity with calibrating beads (Cytometer Setup and Tracking from BD Biosciences). Whole BM was estimated using combination of surface and intracellular staining CD38/CD56/CD138/cytKappa. The sensitivity of our panel MRD is 0.01% (i.e. 10^-4).

Results: Among patients in CR (n=20) confirmed the absence of MRD in 6 patients, but 14 CR patients were MRD positive. MRD was detected in all patients with PR and resistant disease (n=31). The relative content of abnormal plasma cells in CR patients with MRD positive (n=14) was significantly lower than that in PR/resistant patients (n=31): 0.085 (0.026-0.271%) versus 1.3% (0.203-5.9%), pU=0.000092. PR patients (n=8) had a lower relative content of abnormal plasma cells (as expressed tendency), than patients with resistant disease (n=15): 0.286% (0.177-1.129%) versus 1.48% (0.9-0.8%), pU=0.053. Besides the relative content of abnormal plasma cells in PR/resistant patients (n=31) correlated with the serum M-gradient concentration (r=0.42, p=0.019) and the lower dose of GCSF (r=0.54, p=0.0017).

Summary/Conclusions: Currently, we can conclude that MFC could be considered as the method of choice for MRD monitoring in MM. If the disease is measured, then, indeed, enough to evaluate only the M-gradient level of serum. If the M-gradient is not defined, it is necessary to assess the number of abnormal plasma cells in the BM and strive for the high-quality responses at the time of transplantation. And also it can help us to regulate duration of maintenance therapy.
Results: Overall we analyzed 36 pts; 21 males and 14 females (median age 66, range 65-70); 23 had IgG MM, 4 had IgA MM and 9 had light chain MM. Induction therapy was bortezomib-based (bortezomib in combination with dex- amorethasone, RD, in 7, or VD plus thalidomide in 26 pts) for a median of 4 cycles (range 3-6), 2 patients received thalidomide plus dexamethasone (6-12 cycles). PBSC were collected after high-dose cyclophosphamide (2 g/sqm in 2 pts, 3 g/sqm in 11 pts, 4 g/sqm in 22 pts) plus G-CSF, plerixafor was administered in 4 pts. Three pts also received lenalidomide and dexamethasone to improve the depth of response before ASCT. At the time of conditioning, among 34 evaluable pts, 8/34 pts were in complete response/ stringent complete response (CR/sCR), 19/34 in very good partial response (VGPR), 5/34 in partial response (PR) and 2/34 in stable disease (SD). The conditioning regimen consisted of melphalan 140 mg/sqm in 11 pts or 200 mg/sqm in 24 pts. A median number of 4.11 x10^6 CD34+ cells/Kg was reinfused (range 2.09-10.44). The most frequent complication was fever (9 pts) with gram negative bacteremia documented in 3/9 and gram positive bacteremia in 1/9. Other complications were represented by 1 case of atrial fibrillation and 3 cases of pneumonia and 1 case of VZV reactivation. All 35 pts achieved neutrophils recovery after a median of 12 days (range 8-25) and platelets recovery after a median of 13 days (range 8-45) after transplant. No grade 3-4 toxicities were recorded. No transplant-related mortality was recorded within 100 days post transplantation. Three patients died after 28 days (range 18-42) after ASCT. In PR, 14/28 pts in VGPR and 4/28 pts in PR. Three pts underwent tandem ASCT. After a median follow-up of 32 months (range 3-96) among 33 evaluable pts, 20 experienced disease relapse and 7 deaths occurred. Median PFS and OS were 21 and 40 months.

Summary/Conclusions: Our data support the use of ASCT as an effective and safe first-line treatment approach also in elderly MM pts. A careful patient selection is needed to reduce the toxicity of the procedure.

PB1984

EVOLUTION IN THE INCIDENCE OF MONOCLONAL GAMMAPATHIES IN A SOUTHERN SPAIN TERTIARY HOSPITAL IN THE LAST THIRTEEN YEARS

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Background: Monoclonal gammopathy (MG) is the most common plasma cells disorder. It affects around 3% of the population older than 50 years. The great majority of MG are monoclonal gammopathies of undetermined significance (MGUS), which is a premalignant disorder defined to present less than 10% of clonal bone marrow cells and absence of end-organ damage. MGUS is easily detected in laboratory tests and should be monitored because 1% of MGUS per year progress to Multiple Myeloma (MM). The incidence of MGUS and MM is not always easy to determine, but there is a general perception of an increasing incidence that can be attributed to different causes. One is the aging of the population. Another reason is the contribution of clinical laboratories, which count on new determinations (free light chains) or improved techniques in electrophoresis, nephelometry and immunofixation, allowing them to support the diagnose of MGUS and MM.

Methods: In a retrospective study, we determined the total number of MG and MM in a tertiary hospital in southern Spain between 2003 and 2015. We calculated the incidence per 100.000/year of MGUS and MM, with 95% confidence intervals. Our reference population, in 2015, was 480.51.

Results: Results in Figure 1.

Summary/Conclusions: The aging of population and the higher sensitivity of laboratory techniques for diagnosing MG are reflected in the incidence of MGUS, which increased from 17.04 cases per 100.000 in 2003 to 35.00. MM incidence in our area did not increased in parallel.

PB1985

CHARACTERIZATION OF A SERIES OF PATIENTS WITH PLASMA CELL LEUKEMIA

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Background: Plasma cell leukemia (PCL) is a rare malignancy characterized by the proliferation of monoclonal plasma cells in the bone marrow and ≥2x10^9 or ≥20% plasma cells in the peripheral blood. It is an aggressive disease, with a median survival of 7 to 11 months. Due to its rarity, it is difficult to design prospective studies or randomized trials in PCL, so collecting and publishing data from the largest number of cases is essential for the understanding of PCL’s pathophysiology and outcome.

Aims: To characterize a series of PCL patients, in order to obtain data with the potential to be used as prognostic factors and to improve clinical outcomes.

Methods: Single-center, observational, retrospective study including all PCL cases admitted in our hospital between 2007 and 2016. Data regarding demography, clinical characteristics, laboratory results, treatment, follow-up and mortality were collected and analyzed using Statistical Package for Social Sciences (21st version), searching for significant associations (p<0.05) with overall survival (OS) and progression free survival (PFS).

Results: 15 patients were included, with a median age of 58 years. Most patients were male (60%) and had PS ECOG 0-1 (93.3%) at presentation and primary PCL (80%). Median hemoglobin (Hb) and platelets values were 8.5 g/dl and 74x10^9/L, respectively. Median plasma cell percentage was 37.3% (peripheral blood) and 60% (bone marrow). IgG heavy chain was present in 33.3% and lambda light chains in 53.3% of cases. Most patients had total serum calcium ≥4.5mmol/L (60%), total proteins ≥65g/L (66.7%), monoclonal component ≤ 30g/L (53.3%), albumin ≥35g/L (60%), creatinine clearance ≥50ml/min (66.7%), elevated β-2 microglobulin (93.3%), ISS III (80%), R-ISS III (73.3%) and at least 1 cytogenetic change associated with poor prognosis in multiple myeloma (86.7%). Ten (66.7%) patients received bortezomib-based chemotherapy and nine patients (60%) received lenalidomide-based chemotherapy. Complete response (CR) or very good partial response (VGPR) were achieved, after chemotherapy, in 53.3% and, after ASCT, in 88.9% of patients. Mortality rate was 66.7%, with median PFS of 5 months and median OS of 4 months. In univariate analysis, OS was significantly associated with albumin ≤ 35g/L, splenomegaly and R-ISS III; PFS was significantly associated with platelets ≤100x10^9/L, splenomegaly and lambda light chains. In multivariate analysis, only the presence of splenomegaly kept its association with OS; none of the characteristics associated with PFS kept their significance. Chemotherapy followed by ASCT and the achievement of, at least, VGPR after chemotherapy and ASCT were associated with longer OS and PFS.

Summary/Conclusions: This study’s retrospective design and the small sample size limit the strength of our data and our conclusions. Interesting results were obtained regarding pre-treatment prognostic characteristics and the association of improved OS and PFS with treatment response and ASCT execution. More studies are necessary to determine the clinical relevance of this findings and the best treatment strategies in PCL.

PB1986

OPTIMIZATION OF POMALIDOMIDE PLUS LOW DOSE DEXAMETHASONE IN REFRACTORY/RELAPSED MYELOMA MULTIPLE PATIENTS

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Background: MM-003 study has presented a median PFS of 4.0 months and median OS was 13.1 months overall for Pomalidomide and low doses of dexamethasone in RRMM patients. Those results were better when a third drug was added (Poma-Dexa, Poma-Cyclophosphamide-dexa, and Poma-Bortezomib-dexa, ORR 38.9, 64.7 and 85%; PFS 4 4.9, 9.5, 10.7 months respectively).

Aims: We want to evaluate the response at therapy with pomalidomide plus dexamethasone in RRMM, and to analyze the efficacy of another drug in high risk MM.

Methods: We reported the clinical experience of the 8 patients treated with pomalidomide and dexamethasone. In patients with high risk MM (cytogenetic, extramedullary myeloma or plasmatic cell leukemia) pomalidomide and dexamethasone have had poor response. In myeloma in the bone marrow and third drug (cyclophosphamide or Bortezomib) and we have obtained the best results.

Results: We have used pomalidomide and dexamethasone in 4 patients and poma-dexa-cyclophosphamide in 3 patients (extramedullary myeloma) and
poma-bortezomib-dexa in 1 PCL patient. Table 1. Demographic characteristic’s patients. Figure 1. Response of monoclonal spike.

Figure 1.

Summary/Conclusions: pomalidomide, dexamethasone and a third drug (cyclophosphamide or Bortezomib) obtain best results (PFS and OS) in high risk RRMM patients. We have not reported more toxicity adding a third drug. In our experience, the response of the extramedullary myeloma with pomalidomide’s triplets is a great option.

PB1987

PROGNOSTIC SIGNIFICANCE OF PLASMABLASTIC PLASMA CELLS IN THE ERA OF NOVEL AGENTS IN MULTIPLE MYELOMA

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Background: Plasmablastic (PB) feature of plasma cells in multiple myeloma (MM) has long been identified as poor prognosis. Interestingly it does not take part of International Revised Scoring System (R-ISS). Similarly, the prognostic impact in the era of novel agents and novel classes in MM is unknown. Finally, the percentage of PB in the bone marrow to which a poor prognosis develop is unclear.

Aims: To assess which modality of treatment of plasmablastic MM was associated with longer progression free survival (PFS) and overall survival (OS).

Methods: We have performed a retrospective analysis of all MM in our center from May 2005 to November 2016, and sought for MM with plasmablastic features, characterized by immature cells with high proliferative index rate. The PFS and OS were calculated since the first time the PB morphology was observed in the bone marrow aspiration, at the outset in newly diagnosed patients or in relapsed patients.

Results: 65 patients with PB were included. Adverse cytogenetic per IMWG criteria was reported in 6 patients, del(17p) x3, t(4;14) x3, and one with both. 33.8% of the patients were ISS 3, and 23.1% R-ISS 3. Extramedullary disease (EMD) was reported in 6 patients, del17p x3, t(4;14) x3, and one with both. 33.8% of the patients underwent MRI (m:f 51%:58% vs 25%:27%) and skeletal survey (SS) (78% vs 60%) on diagnosis on the AMR than the MRDR, respectively. Baseline laboratory investigations were similar, however, patients on the MRDR demonstrated higher median LDH (m:f 176:178 vs 187:186 units/L) and serum calcium (m:f 2.41:2.45 mmol/L) but decreased serum albumin (m:f 39:39g/L vs 35:35g/L) when compared to the AMR. STAGE: ISS staging was similar on both registries with ISS stage 2 being most common in both cohorts (m:f 42%:37% vs 40%:40%, on the AMR and MRDR, respectively) while ECOG performance status at diagnosis was lower in the MRDR cohort (ECOG1 m:f 43%:44% vs 81%:78%, on the AMR and MRDR, respectively).

Result: First line therapy was predominantly bortezomib (Velcade - V) based on both registries (81% vs 85%). Dexamethasone (D) was the most common on the AMR (29%) followed by V/Thalidomide/D (VTD) (25%) with VCD (79%) on the MRDR, respectively. V was predominantly administered subcutaneously on both registries (79% vs 88%) but more commonly weekly on the MRDR (51% vs 67%) versus twice weekly on the AMR (40% vs 27%). RESPONSE TO THERAPY: Overall response rates were similar between the two cohorts but with higher CR rates on the AMR (CR 21% vs 11%, VGPR 27% vs 31%, PR 31% vs 43%, SD 12% vs 14% and PD 8% vs 2%, on the AMR and MRDR, respectively).

Summary/Conclusions: This pilot study between the AMR and ANZ MRDR demonstrates many similarities but also highlights significant differences, particularly in first line therapy and depth of response. Future studies between the AMR and MRDR will provide a platform for ongoing international benchmarking.

PB1988

INTERNATIONAL OPPORTUNITIES TO COMPARE ‘REAL WORLD’ DATA FROM MYELOMA REGISTRIES: BASELINE CHARACTERISTICS, FIRST-LINE THERAPIES AND EARLY OUTCOMES FROM AUSTRIA AND AUSTRALIA/New Zealand

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Background: Most outcome data for multiple myeloma (MM) come from clinical trials which can not necessarily be extrapolated to ‘real world’ patients. More information is needed on patients treated in the ‘real world’ and in a wider range of settings.

Aims: To compare and contrast baseline characteristics, investigations, and initial therapies in different geographical regions, Australia/New Zealand (ANZ) and Austria, through first analysis of data from two established MM registries on behalf of the steering committees of the Australian and New Zealand Myeloma and Related Diseases Registry and the Austrian Myeloma Registry.

Methods: Analysis of data from newly diagnosed MM patients enrolled on the Austrian Myeloma Registry (AMR) and the ANZ Myeloma and Related Diseases Registry (MRDR) from 2002-2016.

Results: Available data from 250 and 691 patients from the AMR and ANZ MRDR, respectively, were included. DEMOGRAPHICS: The AMR cohort was younger (median age m:f 63.5 yrs:64 years vs 65 yrs:66 yrs on the AMR and MRDR, respectively). The proportion of female: male patients was similar between the AMR and MRDR (m:f 56%:44% and 61%:39%, respectively). PRESENTATION: IgG myeloma was the most common subtype in both disease registries (m:f 64%:55% and 55%:58%, respectively) with more light chain only disease on the AMR (m:f 26%:33% vs 20%:19%). Presence of documented preceding plasma cell dyscrasias was similar (m:f 21%:19% on the AMR and MRDR, respectively). INVESTIGATIONS: A higher proportion of patients underwent MRI (m:f 51%:58% vs 25%:27%) and skeletal survey (SS) (78% vs 60%) on diagnosis on the AMR than the MRDR, respectively. Baseline laboratory investigations were similar, however, patients on the MRDR demonstrated higher median LDH (m:f 176:178 vs 187:186 units/L) and serum calcium (m:f 2.41:2.45 mmol/L) but decreased serum albumin (m:f 39:39g/L vs 35:35g/L) when compared to the AMR. STAGE: ISS staging was similar on both registries with ISS stage 2 being most common in both cohorts (m:f 42%:37% vs 40%:40%, on the AMR and MRDR, respectively) while ECOG performance status at diagnosis was lower in the MRDR cohort (ECOG1 m:f 43%:44% vs 81%:78%, on the AMR and MRDR, respectively).

Result: First line therapy was predominantly bortezomib (Velcade - V) based on both registries (81% vs 85%). Dexamethasone (D) was the most common on the AMR (29%) followed by V/Thalidomide/D (VTD) (25%) with VCD (79%) on the MRDR, respectively. V was predominantly administered subcutaneously on both registries (79% vs 88%) but more commonly weekly on the MRDR (51% vs 67%) versus twice weekly on the AMR (40% vs 27%). RESPONSE TO THERAPY: Overall response rates were similar between the two cohorts but with higher CR rates on the AMR (CR 21% vs 11%, VGPR 27% vs 31%, PR 31% vs 43%, SD 12% vs 14% and PD 8% vs 2%, on the AMR and MRDR, respectively).

Summary/Conclusions: This pilot study between the AMR and ANZ MRDR demonstrates many similarities but also highlights significant differences, particularly in first line therapy and depth of response. Future studies between the AMR and MRDR will provide a platform for ongoing international benchmarking.

PB1989

DETECTING EARLY RELAPSE IN MULTIPLE MYELOMA AFTER ASCT: USEFULNESS OF IMMUNEASSAYS

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Background: The Free Light chain immunoassay (FLC) (BindingSite, Birmingham, UK) is part of the mandatory response assessment for MM, the role of the Heavy/Light Chain immunoassay (HLC) is under investigation. Also relapses in MM patients are frequent, autologous stem cell transplantation (ASCT) is the standard consolidation therapy and there is an interest to detect early relapses using immune assays. We hypothesized that the combination of these techniques could permit to detect early biological (non-symptomatic) relapses (EBR) in this setting.

Aims: To analyze the usefulness of HLC and FLC to detect EBR in MM after ASCT.

Methods: Retrospective study was performed following these criteria: all patients diagnosed of secretory MM, in our center, and treated (including ASCT), between May 2011-August 2015; the protocol for follow-up included FLC, HLC, serum and urine electrophoresis (SPE, UPE), with immunofixation (IFX), pre-ASCT, after 12 months.
weeks and every 3 months later (minimum follow-up: 6 months). EBR was defined as 25% on M-protein increase (any amount for patients on CR/SR) and/or ≥20mg/dI FLC increase, and/or 25% involved HLC increase with abnormal ratios. For urine, an increase >500mg/24h of involved free-chain protein.

Results: Fifty-five patients were registered. Median follow-up 47 months. MF ratio: 29/26, mean age 59.5 y (33-71). Immunoglobulin subtype: IgG: Karpass: 41.8% (23), IgG-Lambda: 23% (12), IgA: Karpass: 16.4% (9), IgA-Lambda: 7.3% (4), Bence-Jones-Karpass: 3.6% (2), Bence-Jones-Lambda: 7.3% (4). Durie-Salmon Stage: IA: 15.3% (7), II-A: 32.7% (17), III-A: 44.2% (23), III-B: 9.6% (5), missing-data 3 case. All patients received Bortezomib based therapy and MEL200 as ASCT conditioning. Status pre-ASCT: minimal response: 12%, Partial Response (PR): 50.0%, very-good-PR (VGPR): 28.0%, complete response (CR): 6% and stable response (SR): 4.0%. After ASCT, evaluation reveals that 13.0% achieved SR, 30.4% VGPR and 39.1% PR. During follow-up, 34/50 (68%) patients who achieved at least PR after ASCT, had a clinical relapse/progress, median PFS 41 months (31.5-50.5). EBR were detected in 28 patients, of them 22/34 (64.7%) clinically relapsed patients at median time 8.0 (2-22) months before symptomatic relapse. The EBR were detected by FLCr (36.7%), HLCr (22.7%), FLC+SPE (4.5%), FLCr+IFX (9.1%), FLC+HLC+HLCr (13.6%), FLCr+HLC+SPE+UPE (13.6%).

Summary/Conclusions: Both FLC and HLC are useful tools to detect EBR in more than 50% of patients in our cohort ahead other techniques.

PB1990

EARLY MORTALITY (<6 M) IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS: COMPREHENSIVE INTERVENTION

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Background: Early mortality in the first 6 to 12 months from diagnosis is well recognized in newly diagnosed multiple myeloma (NDMM) patients, with rates in the real-world setting of around 20-30%.

Aims: In a retrospective analysis of the causes of death performed by the end of 2012 we identify 2 different causes in the 2 consecutive periods analyzed. In the first period (1998-2006) the main cause was MM progression and in the second period (2006-12) was secondary to serious infectious complications. Additional analysis were done after it and can identify a patient and a infectious profiles. Main risk factors from the patient were: age (over 75), suboptimal treatment and renal failure (calculated CICr<50 m/l/min). The infectious main occurred in the first 3 months from diagnosis and principally polymicrobial and multiresistant infections.

Methods: After this analysis several measures were taken to reduce this high early mortality: 1) To promote the ambulatory regime both in diagnosis and for the rapid assessment of complications to avoid or shorten income and to reduce these nosocomial-behaviour infection complications. 2) Early initiation of “optimal” anti-myeloma treatment. 3) Get infectious prophylaxis in patients over 75.

Results: 343 pac NDMM were treated between 1998 and 2015 (127 in the first period, 115 in the 2nd: 242 pts before 2013; and 101 in the 3rd period: 2013-2015). The median age at dx was 74 years (39-100). The number of patients died <6m was 77 years. 60 died before 6 months: 55 before 2013 (29 in the 1st period (22.8%) and 26 in the 2nd (22.6) and 5 after 2013 (5.0%). Of these 60, 37 had a severe infectious complication. The main cause of mortality before 2013 was infectious complications, (14 of 28 early death in the first period and 22.8%) and 26 in the 2nd (22.6) and 5 after 2013 (5.0%).

Summary/Conclusions: Infectious complications and progression of MM have been the main cause of early mortality in patients with NDMM. Identifying potential “modifiable” variables and acting on them improves the short-term prognosis of patients with NDMM like: Supportive treatment to prevent infectious complications (avoid unnecessary hospitalization, antibiottical prophylaxis) and rapid access to optimal antiMM treatments. These improvement of short-term

PB1991

FIRST LINE USE OF NOVEL AGENTS BEFORE AUTOLOGOUS SCT HAS A POSITIVE IMPACT ON TIME TO SECOND PROGRESSION AND SURVIVAL IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA UNDER 70 YEARS

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Background: Most clinical trials for multiple myeloma (MM) patients using novel agent-based regimens before autologous stem cell transplantation have shown improvement in response rates and progression-free survival, however they have failed to identify a significant overall survival (OS) benefit.

Aims: Our aim is to analyze the potential impact of initial induction in the feasibility and outcome of subsequent treatment lines and other major factors affecting OS in a real clinical practice setting.

Methods: Newly MM patients less than 70 years of age diagnosed between December 1999 and December 2009 were prospectively registered. Patients were assigned to a first cohort if they received conventional chemotherapy (CC) induction regimen with new agents available at relapse or to a second cohort if received novel agents based first line treatment (NA).

Results: The overall response rate after completing first line treatment for all the 154 eligible patients was 85%, 79% in CC compared to 94% in NA (P=0.012). Very good partial response or better for NA was significantly higher than for CC (39% vs 29%, P=0.012). Patients in NA demonstrated not only a superior median progression-free survival (2.8 years vs 1.6 years, P=0.03) but also superior median progression-free survival from diagnosis to second progression – PFS2 (5.2 years vs 2.7 years, P=0.003). In both cohorts PFS1 and PFS2 represented more than 50% and 80% of life expectancy respectively. It could be hypothesized that CC patients would obtain more benefit than NA patients of second-line therapy, as they would be naive to the novel agents used at relapse, but this is not the case. The use of thalidomide and/or bortezomib induction did not reduce the efficacy of these same agents second line. Indeed, these patients also had the best second responses that also contributed to longer PFS2 periods. After a median follow-up of 6.97 years, clear differences in OS were observed (7.97 years for NA compared to 3.35 years in CC, P=0.001). Despite the fact that better risk patients in the NA group were more likely to remain in first or second response, relapsed and refractory patients in this group still presented longer survivals beyond second relapse than patients in the CC group (Figure 1).

Summary/Conclusions: New agent based first line induction treatments in newly diagnosed MM patients provide benefits beyond first progression free survival that contribute to a significant improvement in OS.

PB1992

SAFETY AND EFFICACY OF NOVEL AGENTS IN VERY ELDERLY MULTIPLE MYELOMA PATIENTS (AGED 80 YEARS OR MORE): A REPORT FROM THE RETE Eematologico EUGLISE

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Figure 1. (large graphic; legends: red: pre2013; blue: post2013).

Figure 1.
Background: Multiple Myeloma (MM) is mainly a disease of the elderly and the very elderly patients (80 years of age or more) comprise one third about of all MM patients. This subset of patients suffer from concomitant disabilities and/or comorbidities and require a different and more individualized therapeutic approach, including the novel agents.

Aims: The aim of our study is to verify safety and efficacy of novel agents with the reliability to maintain a good quality of life and obtain a maximal disease control.

Methods: Patients from 8 Hematology Centers of the “Rete Ematologico Pugliese (REP)” were included in this study. Between January 2011 and December 2016, 71 patients (MF: 42/29) with a median age of 82 years (range 80-91) were diagnosed as newly symptomatic MM. Of the entire study population, 40 (66%) patients showed an ECOG score lower than 2. According to immunoglobulin heavy and light chain isotypes, patients had IgG-k (n=23), IgG-λ (n=16), IgA-k (n=14), IgA-λ (n=6), micromolecular κ (n=8) and λ (n=4) chains. On the basis of ISS, patients were classified as I (n=4) score, II (n=23) and III (n=44) score, respectively. When CRAB features were considered, bone lesions represented the most frequent (n=43, 60.6%) clinical manifestations, while 30 (42.3%) patients showed renal failure and 26 (36.6%) patients were found in CRAB stage I (n=25, 35.3%), II (n=8), III (n=44) score, respectively. When CRAB features were considered, bone lesions represented the most frequent (n=43, 60.6%) clinical manifestations, while 30 (42.3%) patients showed renal failure and 26 (36.6%) patients were found in CRAB stage I (n=25, 35.3%), II (n=8), III (n=44) score, respectively. When CRAB features were considered, bone lesions represented the most frequent (n=43, 60.6%) clinical manifestations, while 30 (42.3%) patients showed renal failure and 26 (36.6%) patients were found in CRAB stage I (n=25, 35.3%), II (n=8), III (n=44) score, respectively.

Results: Based on IMWG criteria, 15 patients (21.1%) achieved a CR, 15 patients (21.1%) a VGPR and 15 patients (21.1%) a PR. Fourteen patients (19.7%) and 12 (17%) patients experienced a SD and a PD, respectively. As second line of treatment, Bortezomib-based regimens (VMP, VCD and VD) (n=45; 63.4%), Lenalidomide (n=44; 62.5%) and Pomalidomide (n=5; 7%). Only 13 patients (18.3%) did not receive any novel agent. Based on IMWG criteria, 15 patients (21.1%) achieved a CR, 15 patients (21.1%) a VGPR and 15 patients (21.1%) a PR. Fourteen patients (19.7%) and 12 (17%) patients experienced a SD and a PD, respectively. As second line of treatment, Bortezomib-based regimens (VMP, VCD and VD) (n=45; 63.4%), Lenalidomide (n=44; 62.5%) and Pomalidomide (n=5; 7%). Only 13 patients (18.3%) did not receive any novel agent.

Summary/Conclusions: We showed that all MM patients can be treated by novel agents independently of the age. Results from our study show that particularly very elderly and frail patients can benefit from these drugs by prolonging their life expectancy and maintaining a good quality of life.

PB1993

BORTEZOMIB-MELPHALAN-PREDNISONE VERSUS AS CYCLICAL TREATMENT FOR VERY ELDERLY PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA


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Background: Although bortezomib-melphalan-prednisone (VMP) therapy is a well-recommended initial treatment for patients with multiple myeloma (MM) who are ineligible for high-dose therapy, it is not clear whether very elderly patients should be treated with VMP in clinical practice, considering the toxicities.

Aims: The purpose of this case-control study was to compare the efficacy of VMP versus melphalan-prednisone or cyclophosphamide-prednisone (MP/CP) as initial therapy for elderly patients.

Methods: We retrospectively studied 233 patients aged 75 years or older with newly diagnosed multiple myeloma between March 2007 and February 2015. One-hundred thirty one patients received VMP and 102 patients received MP/CP regimen were enrolled from 15 institutions throughout Korea.

Results: Patient characteristics were comparable in these two groups. Overall response rate was 70.2% in VMP patients and 48.0% in MP/CP patients (P=0.001). Complete response rate was 22.9% in VMP patients and 7.8% in MP/CP patients (P=0.002). After a median follow-up for survivors of 28.5 months, progression-free survival (PFS) and overall survival (OS) was significantly different between the two groups (PFS, median 11.5 vs 5.6 months in VMP and MP/CP group, respectively, P=0.018; OS, median 34.9 vs 22.8 months in VMP and MP/CP group, respectively, P=0.006). Nonetheless, for 61 patients who were aged ≥80 years, PFS and OS was not significantly different between the two groups (PFS, median 19.6 vs 13.2 months in VMP and MP/CP group, respectively, P=0.376; OS, median 27.8 vs 17.8 months in VMP and MP/CP group, respectively, P=0.443).

Summary/Conclusions: Although VMP therapy was associated with a significant improvement in overall survival among patients ≥75 years, there is no differences for patients aged 80 or older. Frailty and comprehensive geriatric assessment should be incorporated to guide treatment decisions for this population.
Summary/Conclusions: Multiple myeloma patients with concurrent HIV infection that is controlled on HAART tolerate ASCT for treatment of myeloma as well as myeloma patients without HIV infection and have generally good outcomes.

PB1995
FEASIBILITY OF USING GLOBAL FDG UPTAKE IN BONE MARROW TO ASSESS TREATMENT RESPONSE IN MULTIPLE MYELOMA
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Background: Multiple myeloma (MM) is characterized by plasma cell proliferation and expansion primarily in the bone marrow. Modern assessment of MM using FDG-PET has so far been limited to the analysis of focal lesions, requiring subjective interpretation to determine overall disease activity.

Aims: A novel method using CT segmentation to determine global bone marrow activity portrayed by FDG uptake was used to achieve a comprehensive understanding of disease burden in patients with MM before and after therapy.

Methods: Prospective FDG-PET/CT data of 23 MM patients between ages of 50 and 76 (mean=64.3, males=21, females=2) were collected from Odense University Hospital (NCT02187731) and included scans before initiation of treatment and at end of treatment (EOT) two months after high dose chemotherapy with stem cell support. All scans were conducted 60 min after intravenous injection of 4 MBq FDG. Images were analyzed using an iterative thresholding algorithm that delineates a continuous region based on Hounsfield units from the CT data (Osièx software; Pixmeo SARL; Bernex, Switzerland), allowing for segmentation of the total skeleton on a fused PET/CT image. This enabled the quantification of FDG uptake representing the entire skeleton, providing a global SUVmean that considers all bone marrow involvement. Global SUVmean scores were compared before and at EOT using a two-tailed paired t test.

Results: A decrease in marrow FDG uptake was observed at EOT compared to baseline in most patients. The calculated global SUVmean uptake decreased after initiation of treatment in 17 (73.9%) of the cases and increased in 6 (26.1%) of the cases. The calculated global SUVmean uptake before and after treatment (P=0.0053).

Summary/Conclusions: We assessed the effects of treatment in MM patients using a novel technique for global quantification of FDG uptake in the bone marrow and skeleton and found lower global uptake at EOT. However, a limitation of the PET/CT algorithm is that the total bone marrow is excluded. This is in contrast to the total skeleton approach using a novel technique for global quantification of FDG uptake using an iterative algorithm that delineates a continuous region based on Hounsfield units from the CT data. The novel approach allows for segmentation of the total skeleton on a fused PET/CT image, enabling the quantification of FDG uptake representing the entire skeleton.

PB1996
VALUE OF MYELOLOGIC PROGNOSTIC INDICES IN ERA OF NOVEL DRUGS IN TRANSPLANT SETTING
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Background: Despite the era of emerging novel agents, autologous peripheral blood stem cell transplantation remains backbone of myeloma treatment.

Aims: The main aim of our study was to evaluate the role of tandem transplantation in myeloma treatment as well as prognostic indices in era of novel drug treatment.

Methods: We consecutively included all patients transplanted due to myeloma at our center from 2012 to the end of 2016. Patients were treated with either VAD or VAMP(bortezomib) therapy. All patients proceeded to mobilization therapy cyclophosphamide 3g/m² and received pegfilgrastim. Preparative regimen was either MEL 200 for fit patients or MEL 140 for frail and those with severe renal function impairment. Patients treated with VAD who had poor response after autologous transplantation were subsequently treated with bortezomib based therapy. We examined following baseline characteristics: age, proportion of plasma cells in bone marrow biopsy or aspirate, FISH and lactate dehydrogenase (LDH). Additionally, for each patient International Staging System (ISS), Revised International Staging System (ISS-R) and Durie Salmon staging were calculated. Patients with other malignant diseases prior to myeloma diagnosis were excluded. The main outcomes were International Staging System (ISS), Revised International Staging System (ISS-R) and Durie Salmon staging were calculated. Patients with other malignant diseases prior to myeloma diagnosis were excluded. The main outcomes were

Table 1.

Summary/Conclusions: Prognosis in AL amyloidosis is slowly improving with the use of new anti-myeloma drugs and may improve further with more monoclonal antibodies. Therapy requires an early and accurate diagnosis. We do not perform random biopsies of tissues such as fat or gingivae due to low sensitivity. In our hands biopsies of easily accessible tissues such as subcutaneous fat, gingivae or rectum are usually recommended but sensitivity of this approach is low.

Aims: To present our experience with tissue biopsies performed in 62 consecutive patients diagnosed of AL amyloidosis in our center.

Methods: We reviewed all tissue biopsies performed during the study period (2004-2017) in 62 consecutive patients diagnosed of AL amyloidosis at the same center. A bone marrow (BM) biopsy was performed per protocol in all cases. Decisions on biopsies were taken considering organ involvement and accessibility: skin, lymph nodes, lung or tongue biopsies were performed when lesions were seen on clinical or X-ray examinations, cardiac biopsies in the presence of increased NT-proBNP (N-terminal natriuretic peptide) levels and typical echocardiographic findings, kidney biopsies in patients with nephrotic syndromes. Biopsies were stained with Congo Red and read under polarized light with a Texas filter. Subtyping of the amyloid was done using anti-kappa, anti-lambda, anti-TR and anti-A antibodies. If any biopsy was positive for AL amyloid, no further biopsies were performed unless necessary for therapeutic decisions.

Results: A total of 152 biopsies were performed during the study period: see Table 1.

Table 1.
PB1998
A COMPARISON OF CYCLOPHOSPHAMIDE-GLUCOCORTICOIDS AND LENALIDOMIDE-DEXAMETHASONE AS TREATMENT FOR MULTIPLE MYELOMA IN FIRST RELAPSE AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION
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Background: The optimal management of relapsed Multiple Myeloma (MM) with respect to therapeutic combinations and sequence remains controversial and is actively evolving. Many commonly used regimens have not been directly compared. These agents vary widely in cost, and knowledge of their relative efficacy is of particular importance in regions where cancer medicines are publicly funded.

Aims: We sought to compare the efficacy and safety of two commonly used regimens for relapsed MM using historical cohorts from a single transplant center.

Methods: A retrospective observational study was performed between January 1991 and November 2016 to compare the efficacy of cyclophosphamide and dexamethasone/prednisone (Cyclo), or lenalidomide and dexamethasone (Len-Dex) for relapsed MM post autologous stem cell transplant (auto SCT). The primary outcome was Time to Next Treatment 2 (TTNT2), defined as time from first relapse requiring therapy after auto SCT to second relapse requiring therapy. The secondary outcome was overall survival, defined as time of diagnosis to death from any cause. Outcomes were assessed by Kaplan Meier methods and overall differences determined by log rank test. Hazard ratios were calculated for individual treatment groups and compared by univariate and multivariate logistic regression.

Results: A total of 243 patients underwent treatment for MM at first relapse post autologous transplant. Of these, 139 were included in this analysis: 88 Cyclo and 51 Len-Dex. Patient demographics and disease characteristics were similar between each group for age, sex, subtype of MM and ISS Stage (p>0.05). Vincristine, Doxorubicin and Dexamethasone (VAD) was the most common treatment at diagnosis for the Cyclo group (68%), whereas bortezomib-based therapy was the most common for the Len-dex group (76%) (p<0.0001). No differences were observed in overall response rate or depth of response based on induction therapy between both groups. Median time to first relapse requiring treatment after auto SCT was longer for Len-Dex group (68.5 months) compared to Cyclo group (51.2 months) (p=0.0016). The overall survival was 84 months for Cyclo and 75.6 months for Len-dex (p=0.031). In the multivariate analysis, overall survival was not different for Cyclo compared to Len-dex (HR 0.99; CI 0.42 – 2.34; p=0.99). There was no significant difference in rates of hospitalization, infection, or grade 3 adverse events between the two groups (Figure 1).

Figure 1. Survival curves.

Summary/Conclusions: In this observational study of patients with relapsed multiple myeloma post autologous stem cell transplantation, Lenalidomide-dexamethasone was associated with longer TTNT2 compared with Cylophosphamide-glucocorticoids. However, there was no difference in overall survival. Cyclophosphamide is considerably less expensive than the novel agents. In an era where fiscally sustainable care for MM remains a challenge, further prospective studies are required to compare cyclophosphamide with novel agents in the management of relapsed multiple myeloma.

PB1999
CLINICAL IMPACT OF THE PLASMA LENALIDOMIDE CONCENTRATION AND THE ANALYSIS OF ANTI-TUMOR IMMUNE RESPONSE IN NEWLY DIAGNOSED MULTIPLE MYELOMA TREATED WITH LENALIDOMIDE AND DEXAMETHASONE THERAPY
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Background: Lenalidomide (Len) and dexamethasone (Dex) combination therapy is now the standard treatment of multiple myeloma (MM). Len has both a direct effect on MM cells and an immunomodulatory effect and recently many drugs are combined with Ld therapy to expect the synergistic anti-tumor immune response. However, adverse events (AEs) make continuation of Ld therapy difficult for some patients especially for elderly patients.

Aims: To investigate the safe and effective plasma concentration of Len and the anti-tumor immune response change in MM patients treated by Ld therapy.

Methods: Forty patients (18 men and 22 women) were enrolled in this study. Median age was 75.5 years old (range 61-86). Len was administered on days 1–21 of a 28-day cycle, and Dex, on days 1, 8, 15, and 22. The plasma concentrations of Len just before oral administration and 1, 2, and 4 hr thereafter were analyzed by liquid chromatography-tandem mass spectrometry. Before and after Ld therapy, Peripheral blood mononuclear cells (PBMCs) of MM patients were isolated from whole blood by Ficoll-Hypaque density-gradient centrifugation. PBMCs were stained with the fluorescent dye-conjugated antibodies against surface and intracellular antigens and evaluated by multicolor flow cytometry. Intracellular cytokine production of IFN-γ, TNF-α, IL-2 and CD107a molecule was detected after stimulation with PMA/ionomycin for 5 hours in the presence of protein transport inhibitor Golgi stop (BD Bioscience). Analysis was performed using LSR Fortessa (BD Bioscience) and Flowjo version 10.2 software (TreeStar). This study protocol was approved by the Ethics Committee of Akita University Hospital, and all recipients gave written informed consent.

Results: 21 patients showed renal impairment (RI) necessary to adjust initial Len dosage. Adverse cytogenetics of del17p and t(4;14), detected by using fluorescence in situ hybridization, were found in 2 and 4 patients, respectively. The median initial dosage of Len was 15 mg and Dex 20 mg. The overall response rates were 68.6% and the 2-year progression-free survival was 70.8% at a median follow-up of 26.5 month. Grade 3 to 4 nonhematologic AEs were observed only in 8 patients. We estimated the AUC0-24 of Len by using formula as we previously reported (Ther Drug Monit 2014) and the cut-off value of the hematologic AEs was ≥253 (sensitivity 81%, specificity 80%) and non-hematologic AEs ≥3023.6ng•hr/ml (sensitivity 78.9%, specificity 62.5%).

After Ld therapy, naïve subset of CD4 and CD8 T cells and monocyte MDSC reduced significantly. On the other hand, effector memory subset and intracellular cytokine productions of IFN-γ, TNF-α, IL-2, and CD107a of CD4 and CD8 T cells increased significantly (Figure 1).

Figure 1.

Summary/Conclusions: Len can be administered safely even in elderly patients with RI by using the estimated AUC0-24 of Len as a prediction marker of AEs. Enhanced cytokine production and increased memory subset of T cells were observed after Ld treatment.

PB2000
THE ROLE OF EXPRESSION CD56 ON BONE MARROW PLASMA CELLS AND EXTRAMEDULLARY PLASMA CELLS IN PATIENTS WITH MULTIPLE MYELOMA
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haematologica | 2017; 102(s2) | 795

Madrid, Spain, June 22 – 25, 2017
Background: The myeloma cells interact with the bone marrow microenvironment by several adhesion molecules. One of them is CD56 (a neural cell-adhesion molecule N-CAM) — a membrane glycoprotein, a member of the immunoglobulin superfamily, expressed on the surface of malignant plasma cells of patients with multiple myeloma (MM). Decreased expression of CD56 is considered as one of the possible factors, that help tumor cells to spread outside the bone marrow.

Aims: To evaluate the impact of CD56 expression on the rate of overall survival (OS) in MM patients with extramedullary disease (EMD).

Methods: The study included 32 patients with primary MM (17 males, 15 females) 23-77 years old (median value: 52 years old). The disease was diagnosed in accordance with the IMWG criteria (2014). 17 patients had EMD, including 14 patients with soft-tissue plasmacytomas associated with bone and 3 patients with extramedullary foci in the neck area, in the stomach, in the liver. In all cases a tumour biopsy and bone marrow trephine biopsy were performed, that confirmed the presence of malignant plasma cell infiltration. Paraflin block slices from trephine biopsy material and bone marrow biopsy material were used to perform an immunohistochemistry (IHC) analysis with an antibody to CD56. Kaplan-Meier survival curves were generated, statistical analysis was done using the program Statistica ver.10.

Results: In patients with plasmacytomases the IHC analysis of trephine biopsy material showed CD56+ in 59% cases vs 73,4% in patients without EMD. Five-year OS in patients with CD56+ in the bone marrow was 90%, which was significantly higher (p=0,04) than that of the patients with CD56 - 0% with follow-up of 5 to 61 months (median 20 months, Figure 1). Expression of CD56 on the surface of extramedullary MM cells was found in 76,5% patients. OS in the group of patients with CD56+ in extramedullary MM cells and in bone marrow cells was 67% which was significantly higher (p=0,04) than that in the group of patients (n=4) with CD56+ in extramedullary MM cells and CD56- in bone marrow cells - 50%. Simultaneous lack of CD56 in extramedullary MM cells and in bone marrow cells (n=9) was 67% which was significantly higher (p=0,04) than that in the group of patients (n=8) with CD56+ in extramedullary MM cells and CD56+ in bone marrow cells - 50%. Simultaneous lack of CD56 in extramedullary MM cells and in bone marrow cells was observed in 3 patients with 2 of them died from progression in 31 and 51 months. However simultaneous expression of CD56 in extramedullary MM cells and in bone marrow cells was observed in 9 patients with median follow-up of 40 months and 1 patient died of progression after 47 months.

Figure 1. Probability of overall survival in patients depending on CD56 expression in bone marrow.

Summary/Conclusions: CD56 expression in bone marrow plasma cells significantly increases the OS rate in MM patients regardless the presence or absence of plasmacytomases. Double CD56 negativity both in extramedullary and bone marrow MM cells is a poor prognostic factor with high risk of early relapse and death.

PB2001
BENDAMUSTINE-BORTEZOMIB-DESAMETASONE IN THE MANAGEMENT OF RELAPSED AND REFRACTORY MULTIPLE MYELOMA
C. Cerchione1,*, L. Catalano1, A. E. Pareto1, S. Basile1, L. Marano1, I. Peluso1, A. Darelli, Napoli, Italy

Background: Bendamustine is a bifunctional alkylating agent, with low toxicity, proved to be effective in relapsed, refractory and in newly diagnosed Multiple Myeloma (MM).

Aims: It has been evaluated efficacy and tolerance of Bendamustine, in combination with bortezomib-dexamethasone (BVD) in patients with relapsed and refractory MM (rMM), whose prognosis is particularly severe. A regional prospective real-life analysis of patients with rMM who had been treated with BVD as salvage therapy has been performed.

Methods: 56 patients (31 M/25 F, Table 1), with rMM, median age at diagnosis 57.3 years (r. 36-82), median age at start of treatment 61.8 years (r. 37-83) treated with several lines of treatments (median 6, r. 2-11), every refractory to all the drugs previously received (also Bortezomib), received BVD (Bendamustine 90 mg/sqm days 1,2; Bortezomib 1.3 mg/sqm days 1,4,8,11, Dexamethasone 20 mg days 1,2,4,8,11,9,12, Pegfilgrastim day +4) every 28 days, until progression. ISS was equally distributed, and cytogenetic was evaluable in 12 patients, including 1 del13q and one t(11;14). All the patients had previously been treated with schedule containing bortezomib and IMIDs, and 30% had also received radiotherapy. 67% of them had undergone at least to a single autSCT. All patients were relapsed and refractory to last therapies received before BVD, including bortezomib.

Results: According to IMWG, after a median follow-up of 14 months (r.3-26), ORR was 64% (36/56 : 4 CR, 7 VGPR, 16 PR, 9 MR) with 8 PD and 12 patients in SD, which can be considered as an impressive result in this subset of rMM patients. In particular, for 11 patients, BVD was, after having achieved at least a PR, a bridge to second autSCT, and for two patients a bridge to alloSCT. Median time to response was 1.2 months (r.1-3), median OS from diagnosis was 62.7 months (range 6-151), median OS from start of Bendamustine was 9.8 months (range 2-36).

Table 1.

<table>
<thead>
<tr>
<th>Total patients</th>
<th>56</th>
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</thead>
<tbody>
<tr>
<td>Male</td>
<td>31</td>
</tr>
<tr>
<td>Female</td>
<td>25</td>
</tr>
<tr>
<td>Median age, years</td>
<td>57.3 (36-82)</td>
</tr>
<tr>
<td>at diagnosis, (range)</td>
<td>61.8 (41-73)</td>
</tr>
<tr>
<td>at start of BVD, (range)</td>
<td>6.0 (2-11)</td>
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</tr>
<tr>
<td>negative</td>
<td>10</td>
</tr>
<tr>
<td>del13q</td>
<td>1</td>
</tr>
<tr>
<td>t(11;14)</td>
<td>1</td>
</tr>
<tr>
<td>Previous therapies: no. of patients (%)</td>
<td>56</td>
</tr>
<tr>
<td>Bortezomib</td>
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</tr>
<tr>
<td>IMIDs</td>
<td>50%</td>
</tr>
<tr>
<td>AutoSCT</td>
<td>30%</td>
</tr>
</tbody>
</table>

Summary/Conclusions: BVD has shown significant efficacy in a particularly severe setting of patients, relapsed and refractory to all available therapeutic resources, and, in particular cases, it could be considered as a bridge to a second autologous or allogenic SCT.

PB2002
VE-CADHERIN IN MULTIPLE MYELOMA: AN INDEPENDENT PROGNOSTIC FACTOR FOR PROGRESSION-FREE SURVIVAL
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Background: Endothelial damage and perivascular infiltrates are vital in the development of multiple myeloma. Recent studies have found that endothelial dysfunction might be result in multiple myeloma progression and adverse effects of drug implementation. On the other hand, there is a direct correlation between microvesSEL density in multiple myeloma and parameters of disease progression. Endothelial cells participate in inflammatory events leading to atherosclerosis by regulating endothelial cell permeability via the expression of VE-cadherin.

Aims: The study included 32 patients with primary MM (17 males, 15 females) 23-77 years old (median value: 52 years old). The disease was diagnosed in accordance with the IMWG criteria (2014). 17 patients had EMD, including 14 patients with soft-tissue plasmacytomas associated with bone and 3 patients with extramedullary foci in the neck area, in the stomach, in the liver. In all cases a tumour biopsy and bone marrow trephine biopsy were performed, that confirmed the presence of malignant plasma cell infiltration. Paraflin block slices from trephine biopsy material and bone marrow biopsy material were used to perform an immunohistochemistry (IHC) analysis with an antibody to CD56. Kaplan-Meier survival curves were generated, statistical analysis was done using the program Statistica ver.10.

Methods: The study included 32 patients with primary MM (17 males, 15 females) 23-77 years old (median value: 52 years old). The disease was diagnosed in accordance with the IMWG criteria (2014). 17 patients had EMD, including 14 patients with soft-tissue plasmacytomas associated with bone and 3 patients with extramedullary foci in the neck area, in the stomach, in the liver. In all cases a tumour biopsy and bone marrow trephine biopsy were performed, that confirmed the presence of malignant plasma cell infiltration. Paraflin block slices from trephine biopsy material and bone marrow biopsy material were used to perform an immunohistochemistry (IHC) analysis with an antibody to CD56. Kaplan-Meier survival curves were generated, statistical analysis was done using the program Statistica ver.10.

Results: In patients with plasmacytomases the IHC analysis of trephine biopsy material showed CD56+ in 59% cases vs 73,4% in patients without EMD. Five-year OS in patients with CD56+ in the bone marrow was 90%, which was significantly higher (p=0,04) than that of the patients with CD56 - 0% with follow-up of 5 to 61 months (median 20 months, Figure 1). Expression of CD56 on the surface of extramedullary MM cells was found in 76,5% patients. OS in the group of patients with CD56+ in extramedullary MM cells and in bone marrow cells was 67% which was significantly higher (p=0,04) than that in the group of patients (n=4) with CD56+ in extramedullary MM cells and CD56- in bone marrow cells - 50%. Simultaneous lack of CD56 in extramedullary MM cells and in bone marrow cells was observed in 3 patients with 2 of them died from progression in 31 and 51 months. However simultaneous expression of CD56 in extramedullary MM cells and in bone marrow cells was observed in 9 patients with median follow-up of 40 months and 1 patient died of progression after 47 months.

Figure 1. Probability of overall survival in patients depending on CD56 expression in bone marrow.

Summary/Conclusions: CD56 expression in bone marrow plasma cells significantly increases the OS rate in MM patients regardless the presence or absence of plasmacytomases. Double CD56 negativity both in extramedullary and bone marrow MM cells is a poor prognostic factor with high risk of early relapse and death.
enrolled in the study. Diagnosis and staging of multiple myeloma were defined by current clinical practice guidelines. To be achieving remission chemotherapy with bortezomib, thalidomide, dexamethasone, cyclophosphamide, melphalan, and antracyclines was used according to contemporary clinical guidelines. All subjects were at complete or partial remission at baseline. Observation period was up to 12 months. ELISA method for measurements of circulating level of VE-cadherin was used.

Results: Medians of circulating levels of VE-cadherin in subjects without progression of multiple myeloma (n=89) and subjects with progression (n=23) during 12 months were 0.92 ng/ml (95% confidence interval [CI]=0.66-1.19 ng/ml) and 1.77 ng/ml (95% CI=1.47-2.07 ng/ml) (p=0.0002). The best VE-cadherin cutoff value for predicting progression was obtained with an AUC value 0.833 (p=0.0001), the specificity and sensitivity were 77.8% and 61.5% respectively. The presence of high levels of serum VE-cadherin was significantly correlated to a shorter progression-free survival (PFS). In a multivariate analysis along with clinical and biologic prognostic parameters, high serum VE-cadherin level (>1.31 ng/ml) was an independent adverse prognostic variable for PFS (median PFS 9.93 vs (IC=16-11.71) months vs 7.35 (IC=5.75-8.95) months (p=0.02).

Summary/Conclusions: The serum VE-cadherin level is a valuable biomarker for predicting treatment response and an independent prognostic factor for progression-free survival for patients with multiple myeloma.

PB2003
THE UTILITY OF FACS PURIFICATION OF PLASMA CELLS FOR FISH ANALYSIS IN MONOCLONAL GAMMAPATHIES
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Background: Despite the prognostic value of chromosomal aberrations, conventional metaphase karyotyping in monoclonal gammapathies (MG) is often challenging because of the inherent difficulty of obtaining proliferating plasma cells (PC). Interphase fluorescence in situ hybridization (FISH) is a simple, quick and effective technique for the detection of cytogenetic aberrations that can overcome this limitation. However, the signal of interest is frequently diluted by the noise of the mixed cellularity of the sample, originating both false negatives and false positives. Fluorescence-activated cell sorting (FACS) of the target cells enables a focused application of FISH on pathologically significant cells – such as the PC in MG – reducing the confounding noise. This is particularly relevant when the percentage of pathologic cells in the sample is low, such as in monoclonal gammapathy of undetermined significance (MGUS) where, by definition, there are less than 10% PC in the bone marrow.

Aims: This study aims to analyze the utility and effectiveness of FACS purification of PC for the cytogenetic workup of MG by FISH.

Methods: We analyzed all FISH studies performed in our laboratory, in individual patients, on clonal interphase FACS-separated bone marrow PC, between the 1st June 2015 and the 15th September 2016. The probes used in our standard MG panel were del(1p32), ampl(1q21), t(4;14) and del(17p13.1) (TP53 gene) and, starting in April 2016, t(14;16). We previously established 20 000 cells per sample as the minimum (and sufficient) number of cells needed to obtain a confident application of all 5 probes in our lab.

Results: After the exclusion of samples diluted with peripheral blood, we identified 102 patients with FACS separated purified PC. An average of 165 393±270 516 PC were separated per patient, and 98 of the cohort (96.1%) had a sufficient number of cells for the hybridization of at least one FISH probe; all 5 probes were applied in 30% of patients, 4 in 50%, 3 in 12% and 2 in 8%; the median age of the 98 patients with a FISH result was 63.6 years old (37.8 to 87.3), and 56.1% were male; 41.8% eventually received a diagnosis of MGUS and 58.2% of patients; we were able to apply four or more probes in 80% of patients.

PB2004
CLINICAL SPECTRUM AND EVOLUTION OF MONOCLONAL GAMMOPATHY ASSOCIATED NEUROPATHY VERSUS CHRONIC INFLAMMATORY DEMYELINATING POLYNEUROPATHY PATIENTS
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Background: Paraproteinemic neuropathy (PPN) refers to a disorder of the peripheral nervous system associated with a monoclonal gammopathy (MG). It is known that about 10% of idiopathic peripheral neuropathies are of this type. Unfortunately, PPN is often underdiagnosed or confused with chronic inflammatory demyelinating polyneuropathy (CIDP), subsequently leading to inappropriate management. Since progression of neuropathy is associated with possible malignant conversion of underlying monoclonal gammapathy, it is important to recognize underlying hematological conditions.

Aims: We aimed to determine whether the clinical characteristics and course differed in patients with PPN compared to those with CIDP in order to identify factors useful for differential diagnosis.

Methods: This study was carried out at Seoul National University Hospital, which is a tertiary academic center. During the period between January 2005 and December 2016, patients with 1) monoclonal gammapathy of undetermined significance (MGUS), and 2) CIDP were identified. Those with previous history of cancer or autoimmune disease requiring treatment with immunomodulatory agents were excluded from analyses. In the end, a total of 18 MGUS patients and 34 CIDP patients, with complete set of data including clinical physical examinations, electrodagnostic studies, and laboratory test results, were enrolled.

Results: In both groups, males were predominant. IgG MG was most common (55.6%) in our cohort. PPN appeared to be mainly sensory regardless of heavy chain or light chain. Compared to PPN patients, CIDP patients were associated with motor symptoms manifesting as motor weakness (50.0% vs 91.2%, P=0.001) and ataxia (44.4% vs 61.8%, P=0.043) (Table 1). There were equal number of axonal type neuropathy and demyelinating type neuropathy in patients with PPN, and there were no differences in type of neuropathy between various immunoglobulin subclasses. However, demyelinating type PPN was associated with more severe clinical presentations, including more dyesthesias, pain and sensory symptoms. During median follow-up of 49 months, 2 PPN patients developed overt hematologic malignancies: 1 case of Waldenstroem macroglobulinemia and 1 case of AL amyloidosis. Among them, 2 cases exhibited malignant transformation within 8 months of neuropathy development, and were associated with worsening neuropathic symptom at the diagnosis of hematologic malignancy. There were no differences between the two groups with regards to overall survival.

Table 1. Clinical characteristics of all enrolled patients.
Summary/Conclusions: Although both PPN and CIDP patients suffer from similar symptoms, CIDP patients were more often associated with superimposed motor symptoms. Among PPN patients, demyelinating type neuropathy seems to be associated with more severe clinical presentations. Worsening of neuropathic symptoms in PPN patients warrants a high level of suspicion of malignant transformation of underlying disease.

PB2005

MOLECULAR GENETIC CRITERIA PREDICTING THE EFFICIENCY OF PERIPHERAL BLOOD HEMATOPOIETIC STEM CELLS TRANSPLANTATION IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Global gains in treatment of MM using auto-PBHSCT testify to heterogeneity of long-term outcomes of transplantation - different term of the achievement and duration of complete remission, progression-free survival (PFS), overall survival (OS). These facts determine individual approach to the approach of PFS PBHSCT.

Aims: Finding molecular genetic criteria of predicting the effectiveness of autologous peripheral blood hematopoietic stem cell transplantation (auto-PBHSCT) for improving of algorithm of multiple myeloma (MM) patients cure at various stages of treatment.

Methods: The study involved 61 patients with MM and relapse and primary therapy resistant patients. Molecular cytogenetic, immunogenetic, hematological and statistical methods were used.

Results: Since appearance of genetic abnormalities in the malignant plasma cells is one of the pathogenetic mechanisms of the disease, genetic support of patients is essential. It was determined that the carriage of the allele HLA-DQB1*03: 02 in MM patients is associated with a high risk of high-dose chemotherapy resistance (F=4.83, p=0.028; OR=1.75, p=0.038), and achieving remission after auto-PBHSCT is associated with a carriage of haplotype HLA-C*06 - HLA-DQA1*01: 01 (F=4.87, p=0.028; OR=7.34, p<0.05). Abnormalities of chromosomes 4, 11, 13, 14 and 17 were determined in 35 of 61 (57%) MM patients with complicated disease course and minimal therapy response. Significant alterations were revealed in the presence of two or more abnormal clones (23 patients (37.7%), Ro Spirman=0.42, p<0.05), deletion of chromosome 17 (17 patients (27.9%), Ro Spirman=0.41, p<0.05), deletion/monosomy of chromosome 13 (10 of 15 patients surveyed, Ro Spirman=0.33, p<0.05), the translocation t(4;14) (4 patients (6.6%), Ro Spirman=0.50, p<0.02).

Summary/Conclusions: The results indicate the necessity of introducing the molecular genetic support into protocol of examination MM patients on various stages of treatment with auto-PBHSCT.

PB2006

THE INFLUENCE OF MINIMAL RESIDUAL DISEASE AND TUMOR LOAD ON THE PROGRESSION FREE SURVIVAL IN MULTIPLE MYELOMA PATIENTS

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Background: Use of modern drugs and their combinations in the complex antitymoma therapy (induction, high-dose therapy (HDT) with autologous stem cell transplantation (ASCT), consolidation and maintenance therapy) to improve efficacy of treatment and duration of responses. Despite the achievement of complete response (CR) many patients has a relapse which is caused by activation of residual clonal plasma cells.

Aims: To define influence of induction therapy regimens, HDT with ASCT to the frequency of Minimal Residual Disease (MRD) negative status and estimate a role MRD in duration of Progression Free Survival (PFS) in multiple myeloma (MM) patients.

Methods: We analyzed 52 patients with MM (median age 55 years, male/female – 2:1). The induction therapy with Bortezomib-based regimens (VD, OVD, VMP, PAD) was used in 36/52 (69%) patients, Immunomodulator-based regimens (Thal+D, RD, VRD, PomD) – in 14/52 (27%), chemotherapy – in 2/52 (4%). ASCT is carried out 31 (59.6%) patients. Primary tumor cells phenotype and MRD were detected by 5-color flow cytometry. Clonal plasmatic cells were detected by markers: CD38, CD138, CD45, CD19, CD20, CD27, CD3, and CD138. In 2017, MRD-negative status considered in identifying less than 1 cancer cell in 10000 (0.01%).

Results: MRD-negative CR was reached in 23.8% (10/42) patients after 4-6 cycles of therapy. The frequency of MRD-negative status in the “Bortezomib group” was 31% (8/26), in the “Immunomodulator group” - 7.7% (1/13) (Chi-square p=0.1; p > 0.5). The general frequency of MRD-negative CR after HDT with ASCT was 33.3% (7/21). The carrying out HDT with ASCT allowed to MRD eradication in 36.4% (4/11) patients. One patient with a “light chain” myeloma lost MRD-negative CR after HDT with ASCT that led to development of a clinical relapse after 6 months. Carrying out a maintenance therapy with bortezomib or lenalidomide didn’t allow to achieve MRD-negative status in patients with CR positive response. On the contrary, achieve MRD-negative status promoted to increase of PFS. The PFS median in MRD-negative group of patients (n=36; 21 CR, 6 VGPR, 9 PR) was 21 months, in the MRD-positive group (n=16) – 48 months (p=0.008). The PFS median in patients with CR was higher in the MRD-negative group than in the MRD-positive group (66 and 48 months, respectively, p=0.0045). The tumor load is also a strong prognostic factor like MRD status. Patients who attained low-level MRD had benefit in the duration of PFS: <=0.01% - 66 months, 0.01%-0.1% - 48 months at 0.1%-1% - 22 months, >1% - 10 months (p=0.0009) (Figure 1).

Figure 1. The influence of tumor load on progression free survival.

Summary/Conclusions: The frequency of achievement MRD-negative doesn’t depend from program of induction therapy, HDT with ASCT and maintenance therapy. Negative prognostic role of MRD status independent from clinical response. Presence of MRD after treatment to associated with decrease of PFS and early relapse. Control of MRD allows to increase of PFS and can be done by means of modern drugs and its combinations. HDT with ASCT and maintenance therapy. Impact of MRD requires further studies, especially after HDT with ASCT.

PB2007

QUALITY OF RESPONSE AS PREDICTOR OF SURVIVAL AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN REAL LIFE MULTIPLE MYELOMA PATIENTS IN A SINGLE INSTITUTION

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Background: High dose chemotherapy (HDC) followed by autologous stem cell transplantation (ASCT) is the standard treatment approach for younger patients with multiple myeloma (MM). Since the introduction of proteasome inhibitors and immunomodulatory drugs in MM treatment more patients achieve deep and durable responses and better disease control before ASCT.

Aims: To evaluate the association between the depth of response before ASCT and survival outcomes in a cohort of patients with MM.

Methods: Retrospective analysis of patients with MM treated with HDC and ASCT between 2007 and 2016 in a single institution. All patients received the same stem cell support after conditioning with high dose melphalan (200 mg/m2 and 140mg/m2 for patients with renal insufficiency). Response was assessed 100 days after ASCT according to the International Myeloma Working Group response criteria. The Kaplan-Meier method was used to estimate progression free survival (PFS) and overall survival (OS) and comparison between risk groups was performed by using the log-rank test. The prognostic factors of survival were analyzed by Cox regression univariate and multivariate analysis.

Results: We included 195 MM patients, mainly males (57.9%) with a median age at ASCT of 61 years (28-71). The most prevalent subtype was IgG k (44.1%). The median number of previous therapeutic lines was 1 (1-4) and the majority of patients (61%) received bortezomib as part of first-line regimen. Patients undergone ASCT within a median of 10 months after diagnosis. With a median follow-up time from ASCT of 28.55 months (2.8-121.4), OS at 2 and
Five (29.4%) patients presented with leptomeningeal infiltration; in three of them it was diagnosed at the time of the diagnosis of PCL. All the patients had neurological features. Thirteen (76.4%) patients were able to start a curative treatment: VD in 7 (53.8%) patients, VTD in 2 (15.4%), VAD in 1 (7.7%), D-PACE in 1, MTX-ARAC in 1 patient and RD in the remaining one. Three patients received intrathecal treatment. The intention-to-treat response was: 2 (15.4%) CR, 2 PR, 1 (7.3%) refractory disease, progression and 2 non-evaluable. Only 2 (15.4%) patients achieved enough response (2 CR) to undergo an autologous stem cell transplant (ACST) and only 1 to undergo an allogenic-SCT. With a median follow up of 4 months for all the patients included, median of PFS was 3 (CI 95% 0.47-4.76) months and median of OS was 4 (IC 95% 0.47-7.53).}

Summary/Conclusions: Prospective multicenter studies are required to provide a better understanding of the pathogenesis of PCL. Staging procedures should include lumbar puncture or magnetic resonance at diagnosis when extramedullary involvement is suspected. Intrathecal prophylaxis with cytarabine, metrotexate and dexamethasone is not today a standard of care for patients with PCL.

PB2009

MANAGEMENT AND OUTCOMES OF PATIENTS WITH MULTIPLE MYELOMA IN REAL-WORLD SETTINGS IN BULGARIA, CROATIA AND SLOVAKIA

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1Clinic for Hematology, University Hospital Sofia, Sofia, Bulgaria, 2School of Medicine, Merkur University Hospital, Zagreb, Croatia, 3Department of Hematology, Transfusionology, University Hospital, Bratislava, Slovakia, 4Kantar Health, Paris, France, 5Hemetsberger medical services, Vienna, Austria, 6Amgen (Europe) GmbH, Zug, Switzerland, 7Amgen (Europe) GmbH, Vienna, Austria

Background: The multiple myeloma (MM) treatment (Tx) landscape is rapidly evolving, with varying Tx practice patterns and access schemes across countries. However real-world (RW) data describing patient (pt) management, MM Tx use and outcomes in some Eastern European Countries are limited.

Aims: To understand the characteristics, management, Tx patterns and outcomes in pts with symptomatic MM in a RW setting in Bulgaria (BG), Croatia (HR) and Slovakia (SK).

Methods: Data were collected within a cross-sectional (X) and prospective (R) phase of a chart review in 6 countries between June/15 and June/16 by (onco-)hematologists who managed at least 15 pts with MM per month (mo)

PB2008

LEPTOMENINGEAL INFILTRATION SCREENING SHOULD BE PERFORMED IN PATIENTS DIAGNOSED WITH PLASMA CELL LEUKAEMIA

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Background: Plasma cell leukaemia (PCL) is a rare and aggressive plasma cell (PC) disorder characterized by the presence of circulating plasma cells. PCL can either originate de novo (pPCL) or as secondary PCL (sPCL) in patients with relapsed/refractory multiple myeloma (MM). PCL has a more aggressive clinical presentation than MM with a more frequent extramedullary involvement, such as leptomeningeal infiltration. However, because of the low incidence of this entity, most clinical data come from small retrospective studies. Clinical diagnosis criteria of PCL are today under review and the incidence of leptomeningeal infiltration is unknown.

Aims: We aimed to study the clinical features with special emphasis in the incidence leptomeningeal infiltration in patients diagnosed with PCL in our centre.

Methods: Seventeen patients were diagnosed of PCL between 2008 to 2016 in our centre. PCL was defined based on criteria from the Chronic Leukaemia Group. Median age at diagnosis was 57 years (range 35-80) and 8 (47.1%) were males. Clinical and analytical features at the moment of diagnosis are recorded in Table 1.

Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age</td>
<td>57 years (range 35-80)</td>
</tr>
<tr>
<td>Sex</td>
<td>8 (47.1%) were males</td>
</tr>
</tbody>
</table>

Results: Seventeen patients were included. Six (35.3%) were pPCL and eleven (64.7%) sPCL. Median age at diagnosis was 57 years (range 35-78) and 8 (47.1%) were males. Clinical and analytical features at the moment of diagnosis are recorded in Table 1.

5 years was 83.8% and 68.9% and PFS was 74.8% and 37.3%, respectively. Before ASCT, 101 patients (51.8%) achieved very good partial response (VGPR) or better (≥VGPR) and 94 patients (48.2%) a partial response (PR). The patients in ≥VGPR presented significantly longer OS (median OS not reached vs 96.9 months, p=0.023) and PFS (58.5 vs 41.2 months, p=0.003) compared with those in PR. At 100 days after ASCT, 107 patients (54.9%) presented ≥VGPR, 79 (40.5%), PR and 7 (3.6%) progressive disease. Two patients were not assessed due to loss of follow-up. The group of ≥VGPR showed superior OS (median OS not reached vs 72.4 months, p=0.023) and PFS (58.5 vs 34.7 months, p=0.007) compared to the PR group. We did not find statistically significant differences in survival of patients who achieved ≥VGPR before or after ASCT. Univariate analysis indicates that depth of response before and after ASCT (≥VGPR vs PR) are significant predictors of OS (HR 0.49; 95% CI 0.31-0.80, p=0.004 and HR 0.49; 95% CI 0.30-0.81, p=0.005) and PFS (HR 0.50; 95% CI 0.27-0.92, p=0.026 and HR 0.49; 95% CI 0.27-0.90, p=0.021). Multivariate analysis, after correction noted that these factors retain their prognostic value after adjustment for age, International Staging System stage and number of previous lines of treatment.

Summary/Conclusions: These findings provide evidence for quality of response as a predictor of OS and PFS after ASCT in patients with MM. Outcome after ASCT seems to be better for MM patients who achieve deep responses (at least VGPR) before or after transplant. Our results support the use of more effective induction regimens in order to improve initial response as this may correlate with higher response rates and survival post-ASCT.
Multiple Myeloma Treatments in 11 to 31 Patients

<table>
<thead>
<tr>
<th>Therapy Types</th>
<th>% Responders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lenalidomide, % (n)</td>
<td>80 (13)</td>
</tr>
<tr>
<td>- Bortezomib-based regimens</td>
<td>29 (5)</td>
</tr>
<tr>
<td>- Chemotherapy-based regimens</td>
<td>20 (6)</td>
</tr>
<tr>
<td>- Thalidomide-based regimens**</td>
<td>8 (1)</td>
</tr>
<tr>
<td>- Other</td>
<td>3 (1)</td>
</tr>
</tbody>
</table>

** Using non-prednisone maintenance

PB2010

SINGLE SHOT MEDIUM DOSE MELPHALAN IN RELAPSED MM PATIENTS: A RETROSPECTIVE, SINGLE CENTER EXPERIENCE

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Background: Multiple myeloma (MM) patients refractory to proteasome inhibitors, IMiDs or both, have an extremely poor prognosis. Moreover, they frequently fail to respond to further therapies, and represent a major challenge in everyday clinical practice.

Aims: With this in mind, we treated 12 patient with relapsed MM with a single shot of medium dose melphalan (60 mg/m2) between October 2010 and January 2016.

Methods: The median age was 72 years (range, 62 – 79) and the median time from initial diagnosis to melphalan treatment was 51 months (range, 24 – 144). Patients were heavily pretreated with a median number of 3 prior lines of therapy. All patients were refractory to the previous therapeutic regimens and had failed to respond or were refractory to regimens containing bortezomib. Seven patients (60%) had previously received at least one IMiD, 8 (67%) autologous stem cell transplantation (ASCT) and 1 allogeneic stem cell transplantation. The patients included in the series were not eligible for any clinical trial available at the Institution. All patients gave informed consent.

Results: All patients had cytopenia (anemia, neutropenia and thrombocytopenia). We observed 3 cases of gastrointestinal toxicity (1 bleeding, 1 subcoliculon, 1 mucusseous colitis), 2 patients with WHO, 3 cases of clinically documented infection (1 Escherichia coli bacteremia, 1 fever of unknown origin, 1 erysipela) and 2 deep vein thrombosis. Response was assessed between six and eight weeks after melphalan therapy. Overall, 10 out of 12 patients had a response (1 complete response, 3 very good partial response, 2 partial response and 4 stable disease) only 2 had progressive disease. Median overall survival was 11 months (range, 2 – 37). 10 of 12 patients relapsed after a median time of 5 months (range: 2-12). Concerning two patients not relapsed, 1 patient died in partial response 8 months after therapy of other causes; 1 patient is still alive, in complete remission 18 months after melphalan. He underwent ASCT and maintenance with lenalidomide.

Summary/Conclusions: Many patients refractory to proteasome inhibitors and IMiDs are probably still sensitive to alkylating agents and could be rescued with medium dose melphalan. We suggest therefore melphalan as a "bridge" strategy for further therapy, particularly in patients needing immediate disease control. Even in this era in which several novel drugs became available, single shot medium dose melphalan could be an affordable and safe therapy, able to control aggressive relapse, and to reduce disease burden prior to targeted therapy.

PB2011

LENALIDOMIDE AT THE DOSE OF TWENTY-FIVE MG EVERY OTHER DAY IN PATIENTS AFFECTED BY MULTIPLE MYELOMA AND RENAL FAILURE: A REAL-LIFE EXPERIENCE

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Background: Lenalidomide, available as oral compound, is an IMiD with both antiproliferative and immunomodulatory activity which is largely used in the management of newly diagnosed, relapsed or refractory MM and as maintenance therapy after autologous stem-cell transplantation. Due to its renal route of excretion, it is mandatory to adjust lenalidomide dose in patients with RI, guided by Creatinine Clearance (ClCr), in order to impede a systemic prolonged exposure that could boost myelosuppression. With normal renal function, lenalidomide reaches its maximal plasma concentration after a median time of 0.6-1.5 h, and it therapy. It severely decreases the daily amount of lenalidomide from 15 up to 5 mg (in patients undergoing dialysis); other studies include a schedule with 10 or 15 mg every other day. However, there is no theoretical assumption against the possibility that protracting the time of full standard doses can be equally effective and tolerated by patients required reduced doses.

Aims: In this report, we describe our retrospective experience on the administration of lenalidomide 25 mg every other day for patients with MM and RI.

Methods: From March 2014 to February 2016, 19 consecutive patients, 11 female and 8 male, with a median age of 63.3 years (range: 49-81) affected by advanced, resistant and progressive MM (median number of previous treatment lines: 3, range : 1-5, all including bortezomib) with concomitant renal failure not in dialytic support (median calculated ClCr 36.4 ml/min, range : 18-66) were treated, after informed consent, with monthly 21-day courses of 25 mg lenalidomide every other day and dexamethasone (20-40 mg on days 1-8-15-22, every 28 days).

Results: Disappearance of urinary light chain and reduction of serum creatinine (complete response) were detected in 7 patients (36.8%); 3 patients (15.7%) had a very good partial response, 3 (15.7%) had a partial response, 4 of them (21.0%) were in stable disease, whereas 2 patients (10.5%) had signs of progressive disease. Overall response ratio was 68.2%. More than half of the patients (11/19, 57.8%) had a renal response (median calculated ClCr 51.5ml/min, range 20-168). Median progression free survival was 4 months (range 3-18 months). No patient experienced grade 4 myelotoxicity; four patients required red cell transfusions for grade 3 anemia. No SAE occurred during treatment.

Summary/Conclusions: Dose adjustment RI-related of Lenalidomide is recommended in most guidelines, but there is not a leading scheme with a proven effectiveness more than others. These preliminary observations point to a significant therapeutic effect of lenalidomide, at the dose of 25 mg every other day for 21 days, in more than half of a small population of patients with advanced MM and renal impairment, with not negligible logistic and economic advantages. However, these results should be validated by controlled studies involving larger number of patients.
Results: From March to December 2016 we admitted 57 pts with RI and monoclonal component (29 F, 28 M, 41-83 yrs range), 20 are known MM pts and 37 de novo pts. We diagnosed 11 de novo MM, 13 known MM with a de novo RI, 12 diabetes related RI, 3 amyloidosis, 16 other causes.

Summary/Conclusions: The implementation of the International Myeloma Working Group Recommendations in a routine clinical practice confirmed its feasibility and utility in the optimal workup of MM pts. We obtained diagnosis of RI within 4 days, both in known and in de novo MM pts, with a positive impact on reduced hospitalization, unnecessary dialysis and steroids overtreatment.

PB2014

NOCARDIOSIS PROVOKED BY NOVEL AGENTS AT RELAPSED MULTIPLE MYELOMA: CASE SERIES

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Background: The proteasome inhibitors and immunomodulatory drugs which are used in MM treatment enhance the risk of infection by several mechanisms. Nocardial infections are rare in Turkey.

Aims: Here, we present three relapsed myeloma cases which developed nocardia pneumonia.

Methods: Case 1: 66 year old man, who has a history of autologous SCT 4 years ago and lenalidomide usage because of IgG kappa type myeloma, has been prescribed bortezomib for the relapse of the disease. He was immunocompromised not only because of the myeloma, and also because of the diabetes and renal failure without dialysis. He was admitted to the hospital because of the productive cough. His lymphocyte count was 3200/mm3 and flow-cytometric analysis showed CD5%:68 and CD20:72. Thorax CT showed 39x39x45 mm mass like lesion. Broncoscopic lavage showed branched bacillus via modified acid-fast and Gram stain. This typical morphological appearance was defined as Nocardia spp. Imipenem cilastatin treatment started and control CT was performed and it showed regression of the infiltration. He was discharged with oral TMP/SMX antibiotherapy. Case 2: 71 year old woman, who has a history of two autologous SCT 12 and 5 years ago because of IgG kappa type myeloma; admitted to the hospital with productive cough during ponazolomide treatment. Her lymphocyte count was 2300/mm3 and flow-cytometric analysis showed CD5%:68 and CD20:72. Thorax CT showed a 7x6x8 mm sized mass like lesion with a cavity. Branched Gram positive bacillus (Nocardia sp.) was detected from bronoscopic specimen analysis, so imipenem cilastatin therapy has been started. She responded well to therapy and was discharged with TMP/SMX antibioticotherapy. Case 3: 72 year old woman, who has a diagnosis of IgG kappa type myeloma and a history of autologous SCT 4 years ago following bortezomib treatment, relapsed 5 months ago. He has been admitted to the hospital with nonproductive cough under the treatment of lenalidomide and dexamethasone. His lymphocyte count was 520/mm3. Flow-cytometric analysis couldn’t be performed. Thorax CT showed 4 cm sized cavity and sputum microscopy showed acid resistant branched bacillus thought to be consistent with nocardiosis. The imipenem cilastatin and TMP/SMX treatment have begun and 12 days later, a acid fast resistant branched bacillus thought to be consistent with nocardiosis. The treatment, relapsed 5 months ago. He has been admitted to the hospital with nonproductive cough during ponazolomide treatment. Her lymphocyte count was 3200/mm3 and flow-cytometric analysis showed CD5%:68 and CD20:72. Thorax CT showed 39x39x45 mm mass like lesion. Bronchoscopy showed branched bacillus via modified acid-fast and Gram stain. This typical morphological appearance was defined as Nocardia spp. Imipenem cilastatin treatment started and control CT was performed and it showed regression of the infiltration. He was discharged with oral TMP/SMX antibiotherapy.

Results: See Table 1 and Figure 1.

Table 1.

<table>
<thead>
<tr>
<th>General features of disease</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>66</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>1290</td>
<td>2210</td>
<td>2210</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>328</td>
<td>328</td>
<td>328</td>
</tr>
<tr>
<td>General condition</td>
<td>Good</td>
<td>Poor</td>
<td>Poor</td>
</tr>
<tr>
<td>Myeloma Type</td>
<td>IgG</td>
<td>IgA</td>
<td>IgG</td>
</tr>
<tr>
<td>Precipitating Treatment</td>
<td>Lenalidomide</td>
<td>Lenalidomide</td>
<td>Lenalidomide</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>1 mg</td>
<td>1 mg</td>
<td>1 mg</td>
</tr>
<tr>
<td>Prior lines of treatment</td>
<td>Lenalidomide</td>
<td>Lenalidomide</td>
<td>Lenalidomide</td>
</tr>
<tr>
<td>Relapse Index BX</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Months since last line of treatment</td>
<td>12 month</td>
<td>12 months</td>
<td>12 months</td>
</tr>
</tbody>
</table>

Summary/Conclusions: The proteasome inhibitors and immunomodulatory drugs which are used for the treatment of MM make T cell dysfunction and considering B cell disfunction is also present because of the nature of the disease; this situation tends to provoke rare opportunistic infections such as nocardiosis. Thus, in these patients, it is significant to follow the lymphocyte count closely and to keep in mind that kind of rare microorganisms.

PB2014

LENALIDOMIDE IN PATIENTS WITH DIALYSIS-DEPENDENT END STAGE RENAL FAILURE (ESRF) AND MULTIPLE MYELOMA

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Background: Lenalidomide is an oral immunomodulatory medication with clinical efficacy in relapsed/refractory and treatment naive multiple myeloma (MM),Sq- myelodysplasia and lymphoma. Lenalidomide is eliminated predominantly unchanged by urinary excretion. Renal impairment is common in MM (15-40%) and approximately 10% of MM requires dialysis. However, there is a paucity of clinical safety data of Lenalidomide in ESRF. There is evidence that Lenalidomide can be safely used in patients with moderate and severe renal dysfunction with dose adjustment. However, published data in hemodialysis-dependent patients is limited to a handful of patients across small retrospective analyses and case reports. Patients with ESRF have generally been excluded from clinical trials investigating Lenalidomide. Phase III trials in the relapsed setting (MM-009, MM-010) excluded patients with a serum creatinine >22 µmol/L. The FIRST trial (MM-020), investigating upfront use, excluded patients dependent on dialysis. There is no accepted clinical standard on the most appropriate dosing of Lenalidomide in dialysis. The manufacturer has provided guidelines, being 5mg daily, day 1-21, every 28 days (equivalent to 105mg per cycle). There is alternate well-cited pharmacological dosing that the more appropriate starting dose is likely 15mg per week per cycle, given post-dialysis (equivalent to 135mg per cycle). Aims: To provide real-world evidence of an institutional experience of the use of Lenalidomide in dialysis-dependent MM.

Methods: We performed a retrospective audit of our in-centre experience with treating dialysis-dependent MM with Lenalidomide and included patients who completed at least one cycle of therapy. Patients were assessed for haematological toxicity, significant infective complications, thrombosis, disease response and progression-free survival. Best response was stratified by IMWG criteria.

Results: We identified 5 patients treated between 2010 and 2017, aged between 54 to 73 years old. All patients had relapsed/refractory MM and dialysis dependent ESRF. The median number of prior therapies was two. One patient had (11,14) on FISH and died from progressive disease. Dose schedules are shown in the Table 1. Almost all patients experienced grade III-IV haematological toxicity and 60% had grade III-IV infection. There was a positive correlation between dose and toxicity, and furthermore there appeared to be an inverse relationship between age and tolerated dose. Haematological toxicities and infection were ameliorated by dose adjustment in most instances. There was no drug related mortality, however one patient died of progressive disease. Four of the five patients were prescribed aspirin thromboprophylaxis, with no proven thrombotic complications seen. Where possible to assess, the ORR was 75% (3/4), with 2 patients achieving a very good partial response (VGPR), 1 partial response and 1 progressive disease. The lowest starting dose in this cohort was 10mg twice/week and the maximum dose was 25 mg three times/week.

Table 1.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Gender</th>
<th>Serum creatinine (µmol/L)</th>
<th>Prior lines of treatment</th>
<th>Relapse Index BX</th>
<th>Months from last line of treatment</th>
<th>Dose of Lenalidomide per week (mg)</th>
<th>Best response</th>
</tr>
</thead>
<tbody>
<tr>
<td>54</td>
<td>Male</td>
<td>125</td>
<td>Lenalidomide</td>
<td>2</td>
<td>12 months</td>
<td>5</td>
<td>VGPR</td>
</tr>
<tr>
<td>60</td>
<td>Male</td>
<td>135</td>
<td>Lenalidomide</td>
<td>1</td>
<td>1 month</td>
<td>10</td>
<td>VGPR</td>
</tr>
<tr>
<td>65</td>
<td>Male</td>
<td>145</td>
<td>Lenalidomide</td>
<td>1</td>
<td>12 months</td>
<td>15</td>
<td>VGPR</td>
</tr>
<tr>
<td>73</td>
<td>Female</td>
<td>150</td>
<td>Lenalidomide</td>
<td>1</td>
<td>1 month</td>
<td>15</td>
<td>VGPR</td>
</tr>
<tr>
<td>57</td>
<td>Male</td>
<td>155</td>
<td>Lenalidomide</td>
<td>1</td>
<td>12 months</td>
<td>15</td>
<td>VGPR</td>
</tr>
</tbody>
</table>

Summary/Conclusions: Our experience builds on the emerging evidence that reduced dose of Lenalidomide can be safely prescribed for dialysis-dependent MM with clinical efficacy. Our cohort most patients took Lenalidomide at 15mg per week, 3 times/week on days of dialysis only. There was significant variation of dose-related tolerability between patients. However, toxicity was manageable with diligent monitoring and dose adjustment.
PB2015

STUDY USE OF 18-F FDG PET/CT SCANNING INTO THE FIRST FOLLOW UP OF PATIENTS WITH MULTIPLE MYELOMA AND ASSOCIATION WITH BIOCHEMICAL RESPONSE

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Background: Positron computed tomography (PET/CT) with 18F fluoro-deoxyglucose-labeled glucose (FDG) is a reliable technique with high sensitivity and specificity for assessing skeletal involvement and recent studies propose it as a method for predicting treatment response in multiple myeloma. Conventionally, the response is measurable by the monoclonal component in both serum and urine and Minimal residual disease (MRD) by flow cytometry has been established as a mandatory tool. The studies are aimed at combining the measurement of paraprotein with imaging tests that help to promptly define response or failure to the treatment.

Aims: The primary endpoint was the correlation of the biochemical response with the FDG PET/CT in a second evaluation after first line treatment. The secondary endpoint was the correlation between MRD and with second FDF PET/CT.

Methods: We included in this retrospective and observational study at University Hospital d’Vall d’Hebron, all patients with newly MM and PET/CT before to start a first line treatment and a second PET/CT when completing treatment. PET/CT were analyzed by the department of Nuclear Medicine with experience to grade the lesions in MM, were evaluated and categorized into positive or negative according to the criteria proposed by Zamagni, et al. The biochemical response was defined according to the standard IMWG response criteria.

Results: Eighteen patients (8 males and 10 females) were untreated MM entered, seven patients were classified with ISS III, fifteen had a good performance status, none presented renal lesion, only 16% had hypercalcemia and 66% showed immunoreactivity. Ten patients were IgG isotype, six were classified as light chains myeloma and two patients were oligosecretors. Seventeen patients had bone marrow infiltration with a median of 42% plasmatic cells. Two patients had an extramedullary plasmocytoma and nine had an anormal ratio of light chains. Seventeen patients were treated with bortezomib-based regimens, (median 5.5 cycles) included VTD, MPV, VLD and VD. After treatment, fourteen patients achieved complete response, two partial response and two had progressive disease. PET/CT was positive in all patients pretreatment, 15 focal lesions, 2 diffuse bone marrow involvement plus focal lesions and 1 involvement of bone marrow alone. Twelve patients had more than 3 focal lesions and two had extramedullary disease. At the end of first line treatment, PET/CT was negative in eight patients (44%) and fourteen had complete biochemical response (78%). 62% of the patients with negative PET/CT showed negative flow minimal residual disease (MRD) and biochemical complete response. Two patients had PET/CT with progression disease and corresponded to a biochemical progression.

Summary/Conclusions: The correlation between PET/CT and biochemical response obtained after treatment was positive in patients with complete response. We found discordant data in two patients with oligosecretory myeloma. No correlation was shown between PET/CT and flow MRD. Are necessary more long term studies that include greater number of patients to confirm that the PET/CT negative is an image technique that could be a tool to follow up patients after the first line treatment added to the evaluation of the biochemical response.

PB2017

A RETROSPECTIVE AND PROSPECTIVE AUDIT OF RADIOLOGICAL INVESTIGATIONS FOR SUSPECTED CASES OF PLASMA CELL DYSCRASIAS/ MYELOMA IN THE ALTNAEGELIN AREA HOSPITAL

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Background: The updated NICE guidelines for diagnosis and management of myeloma (2016) suggests whole-body MRI as first-line imaging for people with suspected myeloma and consideration of MRI/CT/PET in newly diagnosed myelomas to assess for bone disease or EM plasmacytoma.

Aims: Our aims were to ascertain: 1) Our current practice regarding radiological investigation for myeloma (2) Whether additional diagnostic information was gained using CT/MRI imaging (3) Since its release, is the trust compliant with the NICE guidance (4) The estimated cost of meeting the current NICE guidance

Methods: Retrospective and prospective audit included all patients having a skeletal survey performed for suspected multiple myeloma within the Alt-nagelin Area Hospital (AAH). Retrospectively from 10/2/15 until 9/2/16 data was collected using the advanced search feature of the Secta ID57 PACS system. The ‘Reason for examination’ for each study was then analysed and those ordered for reasons other than suspected myeloma were excluded. Each case was analysed individually and any follow up MRI/CT/NMB imaging performed in the 6 month period following the skeletal survey were included in the data collection. The same information was gathered prospectively from 10/2/16-30/5/16 following the NICE guidance. 54 skeletal surveys where performed for suspected myeloma.

Results: The indications for requesting imaging is shown in Table 1A. No WB MRI/CT was performed in this period. 26 patients had new lytic lesions on skeletal survey. 23 patients had further imaging in the form of MRI or CT following skeletal surveys. All the positive MRI findings offered additional diagnostic information - including examples of missed multiple spinal deposits. The results of imaging are summarised in Table 1B. The false negative rate for skeletal surveys was 39% and the false positive rate was 22%. Following NICE guidance publication 23 patients had skeletal surveys performed for suspicion of myeloma between 10/2/16 and 30/5/16. The indications are summarised in Table 1C. No WB imaging was performed. 5 patients had positive skeletal surveys. 6 patients had subsequent CT/MRI imaging. A skeletal survey was report normal with a subsequent MRI showing multiple spinal deposits. The imaging results are summarised in Table 1D.

Table 1

Summary/Conclusions: The expected cost of implementing WB imaging for 60 patients per year in the AAH is £18,240. In comparison the cost of performing skeletal surveys would be £4200 per annum. NICE guidance 2016 offers an...
economic model for imaging with WB MRI. In addition it reviews evidence which links time to diagnosis to survival and myeloma related complications. The NICE guidance offers clear evidence that WB-MRI should be the investigation modality of choice for suspected myelomatous disease. It offers a diagnostic and cost-effective strategy that will ensure health improvements for myeloma patients. This audit offers further evidence of the diagnostic accuracy of MRI imaging. At present failure to comply with NICE guidance will lead to delayed diagnosis of myeloma in certain patients and potential patient harm. Therefore I offer a business and health improvement case for the Western Trust to instigate WB-MRI imaging for all suspected myelomatous bony disease.

PB2018

TONI DEBRE FANCONI SYNDROME DURING MYELOMA, ABOUT 8 CASES
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Background: The cast nephropathy with cylinders is the most frequent renal complication of the myeloma, which results from a catabolism of the light chains by the tubular cells and can lead a tubular chronic suffering showing itself by a syndrome of acquired Toni-Debré-Fanconi marked by a glycosuria, a phosphaturia, an aminoaciduria, a sometimes severe and sometimes revealing hypokalemia.

Aims: We reporting some observations informed by Multiple Myeloma complicated with a Fanconi syndrome.

Methods: From January 2000 till December 2010: 78 cases of Multiple Myeloma were brought together, whose circumstance of discovery 22 cases with renal failure, it's was a evolutes complications in 12 cases; and in 10 cases it's discovered at diagnosis. The renal achievement is dominated by Tubule disease in 11 cases, Randall syndrome 8 cases, and Nephrotic syndrome in 3 cases. The tubule disease of Fanconi is suspected at only 8 patients: in front of the presence of a glycosuria (without associated diabetes) and a frank proteinuria in the majority of the cases, with a hypophosphatemia and a fickle hypokalemia.

Results: The clinico-epidemiological and immuno-biological characters of these 8 patients are the following ones: - The median age is of 64 years (39-76), sex ratio 3. The osseous pains and the muscular cramps dominate the clinical presentation with constant diffuse demineralization in the radiology. - The patients were classified (according to the Salmon-Durie classification): IIIb (3 cases) and IIb (5 cases). ISS 3 in majority of the cases. - The monoclonal immunoglobulin observed: IgG kappa: 4cases, IgA kappa: 2cases, light chain kappa: 2cases. With a Bence Jones proteinuria isotype kappa and a glycosuria in the majority of the cases. - The gravity of the renal failure, based on the clearance of the creatinine: with an average clearance of 16.19 ml/min (4-37): several in 5cases, terminal in 3cases. - We note more of hypocalcaemia while the hypercalcaemia is noted in a single case, the hypophosphatemia is found in half of the cases. The therapeutic management is double: - Symptomatic: alkaline hydration, correction of the metabolic disorders and sometimes the renal extra purge (indicated in 3cases). - Specific: chemotherapies VAD:Cases, a patient died by cardio-vascular complication. Under treatment the recovery of the renal function is obtained in 3 cases, to the rests of the patients persists a stable renal failure.

Summary/Conclusions: The Syndrome of Fanconi is a frequent and often formidable complication during Myeloma, observed to 30-40% of the patients in an autopsie series. It is necessary to think to it in front of any renal achieve-ment in myeloma of kappa light chain with renal glycosuria, a generalized aminoaciduria and a hypophosphatemia resulting respectively from a defect of the transport of the glucose, from amino acids and from phosphates by the renal proximal tubule. To improve the osseous and renal appearances, it is necessary to realize a calcic supplementation, phosphorous and by the vitamin D active, as well as the correction of the acidosis and a specific treatment reduc-ing the excretion renal of the light chains.

PB2019

DEPP RESPONSES WITH CARFILZOMIB-LENALIDOMIDE-DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS: A REAL LIFE EXPERIENCE
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Background: Carfilzomib is a new proteasome inhibitor with in contrast to the reversible binding of bortezomib, binds irreversibly and selectively to its target: the chymotrypsin-like activity of the 20S proteasome. The phase IB/II PX-171-006 study was the first study in which carfilzomib was combined with lenalidomi-dexmethasone. In the phase I dose-escalation part the maximum tolerated dose was established as well tolerated and in the phase II part the study focused the efficacy and toxicity in the subgroup treated with maximum planned dose. The ASPIRE trial showed superior response rates and progression free survival for carfilzomib-lenalidomide-dexamethasone compared with lenalidomide-dexamethasone in relapsed/refractory Multiple Myeloma patients. Aims: The aims is explore the efficacy and tolerability of carfilzomib-lenalidomide-dexamethasone in relapsed/refractory Multiple Myeloma patients in real life.

Methods: All patients received carfilzomib 20/27 mg/m2 days 1,2,8,9,15 and 16; lenalidomide 25 mg days 1-21 and dexamethasone 20 mg days 1,2,8,9,15,16, 22 and 23, according to post approval access protocol. After 2, 4, 6 and 8 cycles the responses, disease progression and toxicity were assessed using the International Myeloma Working Group Uniform Response Criteria and WHO score respectively.

Results: From January 2016 to February 2017 in hematology “Cardinales G.Panico Hospital” and “Bari Policlinico”, treated 15 relapsed/refractory Multiple Myeloma patients with carfilzomib-lenalidomide-dexamethasone. Six patients male (40%), 9 female (90%), mean of age 62 years (range 38-79); 10 (66%) and 5 (34%) relapsed/refractory multiple myeloma respectively. Median time from diagnosis to carfilzomib-lenalidomide-dexamethasone was 46 months (range 12-92); median of prior therapy was 3 (range 1-4); 9 (60%) received autologous transplantation while 1 (6%) prior therapy with lenalidomide; 15 (100%) prior therapy with bortezomib; 7 (14%) prior therapy with pomalidomide (Table 1). Eleven (73%) patients achieved after 2 cycles a response rate ≥PR, of these 3 VGPR. After 4 cycles, 5 (33%) and 1 (7%) have obtained at least a VGPR and CR respectively (Figure 1). Three patients were not evaluated for treatment discontinuation because of rapid progression disease and died during first cycle with a median of 5 prior lines therapy. Most grade 3-4 adverse events were haematological and well manageable, 10 (80%) trombocitopenia and 5 (35%) neutropenia grade 3-4. Dyspnea, fatigue and pyrexia were higher but were mostly grades 1 and 2. Only 2 patients developed respiratory failure and pneumonia while cardiac failure, ischemic heart disease and hypertension not were detected. 

Table 1: Baseline patient characteristics.

<table>
<thead>
<tr>
<th>MEAN OF AGE, years (range)</th>
<th>RELAPSED</th>
<th>REFRACTORY</th>
</tr>
</thead>
<tbody>
<tr>
<td>62 (38-79)</td>
<td>11 (50)</td>
<td>5 (34)</td>
</tr>
<tr>
<td>MULTIPLE MYELOMA, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>4 (40)</td>
<td></td>
</tr>
<tr>
<td>Igκ</td>
<td>2 (20)</td>
<td></td>
</tr>
<tr>
<td>light chain kappa</td>
<td>7 (46)</td>
<td></td>
</tr>
<tr>
<td>MICROBIOLLOGICAL FACTORS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STAGING, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I   -</td>
<td>1 (5)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>III -</td>
<td>3 (20)</td>
<td>12 (80)</td>
</tr>
<tr>
<td>STAGE OF DISEASE, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III -</td>
<td>7 (47)</td>
<td>8 (53)</td>
</tr>
<tr>
<td>MEDIAN TIME FROM DIAGNOSIS TO KID., months (range)</td>
<td>46 (12-92)</td>
<td>3 (6-10)</td>
</tr>
<tr>
<td>MEDIAN OF PRIOR THERAPY, lines (range)</td>
<td>3 (6-10)</td>
<td></td>
</tr>
<tr>
<td>PRIOR TRASPLANT, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUTOLOGOUS</td>
<td>9 (60)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>ALLOGENIC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRIOR THERAPY, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LENALIDOMIDE</td>
<td>11 (73)</td>
<td>15 (100)</td>
</tr>
<tr>
<td>BORTHEZOMIB</td>
<td></td>
<td>3 (14)</td>
</tr>
<tr>
<td>POMALIDOMIDE</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1.

Summary/Conclusions: Carfilzomib-lenalidomide-dexamethasone is a powerful and efficacy association in relapsed/refractory Multiple Myeloma patients, which allows the achievement of deep responses from the first cycle of therapy. Non haematological adverse events of grade 3 or higher were reported in only 2 patients.
PB2020
CARFILZOMIB-LENALIDOMIDE-DEXAMETHASONE IN THE MANAGEMENT OF RELAPSED AND REFRACTORY MULTIPLE MYELOMA: A REAL-LIFE EXPERIENCE
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Background: Carfilzomib is an epoxyketone proteasome inhibitor of second generation, proved to be effective in relapsed and refractory Multiple Myeloma (rMM).

Aims: In this retrospective observational trial, it has been evaluated efficacy and tolerance of Carfilzomib, in combination with lenalidomide-dexamethasone (KRD), as salvage regimen in patients with relapsed and refractory MM (rMM), whose prognosis is particularly severe.

Methods: 21 patients (12 M/9 F, Table 1), with rMM, median age at diagnosis 62 years (r. 47-75), median age at start of treatment 65 years (r. 53-81) treated from Dec-2014 to Feb-2017 (28 months). 55 patients were diagnosed of MM, 26 were male and 29 female. The median age at diagnosis was 74 years (52-87), 11 were under 65 (U65) and 44 were over 65 (O65).

Results: KRD was well tolerated, with grade 2 anemia in 28% of patients, without necessity blood transfusions; 5% grade 1 and 9.5% grade 3 neutropenia (no ospedalization was required); 33% grade 2, 19% grade 3 and 5% grade 4 thrombocytopenia, with hemorrhagic events and necessity of transfusions. Concerning severe extrahematologic toxicity, it was observed grade 1 pneumonia in 47% of patients, treated by common antibiotic drugs; grade 2 Hypertension in 24% of patients; grade 3 arrhythmias in 5% of patients; grade 2 dyspnea in 5% of patients; grade 1 fatigue in 9.5% of patients. According to IMWG criteria, after a median follow-up of 3 months (r.1-13), ORR was 66.7% (14 VGPR, 6 PR) with 3 progressive diseases and 2 patients in stable disease, which can be considered as an impressive result in this subset of rMM patients. In particular, for 1 patient, KRD was, after having achieved at least a PR, a bridge to second auSCT. Median time to response was 2 months (r.1-4), median OS from diagnosis was 47 months (9-170 range), median OS from start of Carfilzomib was 3 months (range 1-13).

Table 1.

PB2021
IMWG 14 DIAGNOSTIC CRITERIA TO INITIATE TREATMENT IN NEW DIAGNOSED MULTIPLE MYELOMA: REAL-WORLD STATISTICS
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Background: Diagnostic criteria for Symptomatic Multiple Myeloma (MM) Published in 2003 by the International Myeloma Working Group(IMWG03) established in the presence of a bone Marrow infiltration by plasma cells (BMPC) in any percentage And / or the presence of a monoclonal component of any amount Along with the presence of signs or symptoms of organ damage (CRAB) attribuable to the proliferation of plasma cells. These criteria have not changed in the last decade until the Recent revision of diagnostic criteria and treatment that IMWG Published by the end of 2014, which proposes an initial Pathologic condition (>10% BMPC or demonstration of a Plasmacytoma as a preliminary condition before starting treatment. Due to “CRAB redefined” and / or the presence of markers of Rapid progression to “classical-symptomatic” MM criteria.

Aims: There are few information about real-life statistics in NDMM according to new criteria to initiate treatment. This 2year analysis shows a percentage of patients (22%) who have initiated new treatments superior to those described in the literature

Methods: We have performed a retrospective analysis with all new MM cases diagnosed from Dec-2014 (after new criteria were published) to Feb-2017 (28 months). 55 patients were diagnosed of MM, 26 were male and 29 female. The median age at diagnosis was 74 years (52-87), 11 were under 65 (U65) and 44 were over 65 (O65).

Results: 3 were diagnosed after biopsy of plasmacytomas. None of them have Bone Marrow (BM) infiltration but with criteria of MM after PET-CT multilocip involvement. 7 of these NDMM were smoldering MM (sMM). All of them completed initial staging with more sensitive imaging tests than conventional MRI (and / or PET-CT) 2 of these sMM were under 65 years old and were included in a clinical trial. The other 5 were older than 65 and after a median of 16 months of follow-up did not meet criteria in initiate treatment. Of the 41 patients who started treatment, 10 of them were new criteria, the rest met criteria for classic organic disease (CRAB) Figure 1. 6 patients were diagnosed after performance of PET-CT (3 of them after plasmacytoma biopsy; initial diagnosis: solitary plasmacytoma), 1 after PET-CT negative but MRI positive, 2 with FLC ratio criterium and the last one with BM Plasmatic Cell (BMPC) >60%, MRI image and FLC criteria. Although these data are quite different from those reported previously, accurate diagnosis in initial stages may increment the proportion of real-active MM. We don’t observe increments in incidence rate in these period vs 2014 (reports from EHA congress). We observe that the early mortality is decreasing in the last 5 years (from 2013). The effect of early diagnostic may contribute to get these improvement of survival.

PB2022
POMALIDOMIDE PLUS LOW-DOSE DEXAMETHASONE: A CHANCE FOR REAL-PRACTICE AND/OR REAL-LIFE MULTIPLE MYELOMA: A REVIEW OF A CASE SERIES DIAGNOSED IN A SINGLE CENTER
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Summary/Conclusions: KRD has shown significant efficacy in a particularly severe setting of patients, relapsed and refractory to all available therapeutic resources, and, in particular cases, it could be considered as a bridge to a second autologous or allogenic SCT.
Background: The treatment of patients with multiple myeloma (MM) has dramatically changed over the past decade due in part to the development of new agents and myeloma-specific targets. Nowadays, new effective treatments exist for patients with RRMM not responding to bortezomib and lenalidomide. Pomalidomide alone has shown limited efficacy in patients with RRMM, but synergistic effects have been noted when combined with dexamethasone.

Aims: To show our experience with the use of 28-day cycles of pomalidomide (4 mg/day on days 1–21, orally) plus low-dose dexamethasone (40 mg/day weekly, orally) (Pom/dex) in RRMM.

Methods: This is a retrospective study performed between May 2014 and January 2017 in the Hospital of Guadalajara (Spain). Eight patients (3M, 5F), with a median age of 67 years (range, 40–81), diagnosed with MM and WM were included. Four were classified as high-risk myeloma (Patients 1–4). Patient 1 (P1) had plasma cell leukemia and received Pom/dex plus bortezomib; Patient 2 (P2) presented complex karyotype and received Pom/dex after three previous regimens and an autologous transplantation; Patient 3 and Patient 4 (P3 and P4) had extramedullary plasmacytoma and received Pom/dex/local radiotherapy. The eight patients of this study had failed to bortezomib and lenalidomide-based therapy, and received Pom/dex until disease progression or unacceptable toxicity. Pom/dex was associated with ciclophosphamide in two patients, and with bortezomib in another two patients. The primary endpoint was progression-free survival (PFS).

Results: The median number of prior regimens was 2 (range, 1–4) and five of eight patients (62.5%) had previously received autologous transplantation. Median time from diagnosis to Pom/dex was 51.5 months (range, 28–155). Patients received a median of 6 cycles of Pom/dex (range, 2–16). In the whole series, the median follow-up was 60.5 months (IQR: 50–60.8), and median PFS was 11 months; 75% of patients had not progressed after 5 months, and 50% of patients after 11 months. The overall response rate was 87.5% (one patient discontinued therapy for non-response). In standard-risk MM patients, median follow-up was 61 months (IQR: 46.25–140.25), and median PFS was 13 months; 75% of patients had not progressed after 2 months, and 50% of patients after 13 months. Regarding the high-risk group of patients, P1 achieved complete response after 2 cycles of Pom/dex/bortezomib; P2 achieved PFS of 11 months; P3 achieved plasmacytoma resolution after 6 cycles of Pom/dex plus local radiotherapy; P4 abandoned Pom/dex after 3 cycles because of severe neutropenia and sepsis. In this group median follow-up was 60.5 months (IQR: 56.3–79.8), and median PFS was 6 months; 75% of patients had not progressed after 5 months, 50% of patients after 6 months, and 25% of patients after 11 months. Regarding adverse effects, they were present in two patients: one had neutropenia, and the second one pneumonia plus pulmonary venous thromboembolism. Both of them died (Figure 1).

Summary/Conclusions: In our experience, Pom/dex regimen has prolonged PFS of patients with RRMM, with an improvement of health-related quality of life. This regimen has been even valuable in high-risk patients who received Pom/dex after ≥2 treatment regimens. Pomalidomide plus low-dose dexamethasone, an oral regimen, could be considered a new treatment option as a standard of care for patients with RRMM who have poor prognosis and a high need for effective treatments.

Myeloproliferative neoplasms • Biology

PB2023

ROUTINE SCREENING FOR KIT M541L IS NOT WARRANTED IN THE DIAGNOSTIC WORK UP OF PATIENTS WITH HYPEREOSINOPHILIA

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Background: The role of the KIT M541L variant in patients with hypereosinophilic (HE) is controversial. On the one hand, this variant is a recognised driver in single nucleotide polymorphism (c.1621A>C; rs3822214) with a minor allele frequency of 0.08 in the ExAC database and classified as benign/likely benign on ClinVar. On the other hand, it has been suggested that KIT M541L increases the sensitivity of the KIT receptor to stem cell factor (Foster R et al., Br J Dermatol. 2008;159:1160-9) and may be somatically acquired in idiopathic chronic eosinophilic leukemia/chronic myeloid leukemia (CEL-NOS) patients negative for PDGFRαβ abnormalities (Lufro A et al., Oncotarget. 2014;5:4865-70). Consequently it has been suggested that HES patients should be screened for KIT M541L, as positive cases may benefit from imatinib treatment.

Aims: We aimed to (i) compare the KIT M541L allele frequency between patients referred for investigation of HE and normal healthy controls and (ii) investigate the variant allele frequency (vaf) to determine if KIT M541L mutations may be acquired somatically and (iii) investigate the KIT M541L status in cases negative for PDGFRαβ abnormalities who responded to imatinib.

Methods: We screened healthy controls (n=214) and patients referred for investigation of FIP1L1-PDGFRα negative HE (n=220) for KIT M541L using an amplification refractory mutation system (ARMS) PCR designed to amplify allele specific products of different sizes, and able to detect KIT M541L down to 5% vaf. Fishers exact two tailed test was used to compare the allele frequency between the HE and control HE groups. Digital droplet PCR (ddPCR) was used for patients heterozygous for KIT M541L to determine whether the KIT M541L mutation burden was close to 50% (consistent with a constitutional polymorphism) or <50% (suggestive of a somatic mutation). We also studied pre-treatment DNA from 3 patients with hypereosinophilic syndrome who were treated with imatinib (400 mg/day) and showed normalization of eosinophil counts at a median of 0.8 months (0.4-5.0) after treatment for a duration of 13.6 months (range, 3.7-44.8).

Results: Forty two (19%) of HE cases tested positive for KIT M541 compared to 38 (18%) of healthy controls. The KIT M541L allele frequency was no different between cases and controls (0.098 versus 0.09, P=0.91). Of the 42 KIT M541L heterozygous HE cases, 40 had sufficient DNA for analysis by ddPCR. The mean allele burden was 50.4% (range 48.3%>56.0%), consistent with all instances being constitutional. None of the three imatinib responders tested positive for KIT M541L prior to treatment.

Summary/Conclusions: Whilst we cannot exclude the possibility that KIT M541L may be acquired somatically in very rare cases, we conclude that there is no clinical value in screening for this variant on a routine basis for patients with HE or HES.

PB2024

MUTATIONS OF THE JAK2 GENE AND CYTOGENETIC ABNORMALITIES ARE PREDICITIVE OF PROGRESSION TO HEMATOLOGICAL NEOPLASMS IN PATIENTS WITH IDIOPATHIC LEUKOCYTOSIS

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Background: Idiopathic leukocytosis and erythrocytosis are hematological disorders without specific causes. Frequent V617F mutations on the JAK2 gene have been reported in patients with polycythemia vera (PV), essential thrombocythemia, and primary myelofibrosis. We also found JAK2 V617F mutations in one of 11 patients with idiopathic erythrocytosis. Mutations of the CSF3R, JAK2, ETNK1, and SF3B1 genes have been found in chronic neutrophilic leukemia and atypical chronic myeloid leukemia (CML). Furthermore an autosomal mutation was found in the CSF3R gene in a family with chronic neutrophilia. However, little is known about mutations associated with idiopathic leukocytosis. Additionally, we previously analyzed the JAK2, CSF3R, CALR, SETBP1, and ETNK1 genes in 10 patients with idiopathic leukocytosis (EHA20). To elucidate the relevance of genetic alterations, we extended the analysis with 17 genes known to be implicated in hematological neoplasms in 16 patients with idiopathic leukocytosis.

Methods: Leukocytosis is defined as a total white blood cell count more than two standard deviations above the median, or a level of neutrophils (лей) greater than 11,000/μL. Those patients who satisfied the following criteria were included in the study: leukocytosis (predominantly neutrophils); the absence of apparent causes of leukocytosis; and documentation of the leukocytosis over a prolonged period without specific causes.
of time. The period of observation was 1 year or longer in most patients. Sixteen patients with idiopathic leukaemia were analyzed in the study. Neutrophils or mononuclear cells were collected after obtaining written informed consent from the 16 patients. Neutrophils from peripheral blood were purified by dextran sedimentation followed by hypotonic lysis and centrifugation with Ficoll-Conray. Mononuclear cells were isolated from bone marrow by Ficoll-Conray gradient centrifugation. DNA was extracted using the QIAGEN DNA blood kit (Qiagen, Valencia, CA, USA). Mutations within hot spots of the CSF3R, JAK2, CALR, SETBP1, ETN1K, CBL, TET2, ASXL1, EZH2, IDH1/IDH2, DNMT3A, U2AF1, and CEBPA genes were analyzed by direct sequencing in both directions using a 3730XL DNA Analyzer (Life technologies, Carlsbad, CA, USA) and/or allele-specific primers. Total RNA extraction and reverse transcriptase polymerase chain reaction (RT-PCR) were performed between the ETF6 and ABL1 genes in 10 patients. BCR/ABL1 gene was analyzed by RT-PCR or fluorescence in situ hybridization in 8 patients. The current study was conducted within the guidelines and with the approval of the ethics committee.

Results: JAK2 V617F mutations were found in one of the 16 patients with idiopathic leukaemia. No mutations were found in the other genes in the 16 idiopathic leukaemia patients. ETF6-ABL1 fusion gene was detected in one of the 10 patients. No BCR/ABL1 fusion gene was detected in the 8 patients. One idiopathic leukaemia patient with JAK2 V617F mutation has developed PV. Another patient with sustained leukaemia for 20 years showed cytogenetic abnormalities during observation and has developed Philadelphia chromosome negative CML (Ph-CML). ETF6-ABL1 fusion gene was detected in this patient. Another patient with normal karyotype progressed to blast crisis of Ph-CML characterized by cytogenetic abnormalities. Of the remaining 13 patients with idiopathic leukaemia, one resolved the disease and twelve had a stable disease.

Summary/Conclusions: Idiopathic leukaemia comprises heterogeneous conditions. JAK2 mutations and cytogenetic abnormalities are predictive of progression to hematological neoplasms.

PB2025

EVALUATION OF EXPRESSION OF MiRNAs ISOLATED MICROVESICLES OF PATIENTS WITH MYELOFIBROSIS ASSOCIATED WITH DISEASE

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22 nd Congress of the European Hematology Association

Background: Myelofibrosis is a hematological disease inserted in the group of myeloproliferative neoplasias. It has as main characteristic fibrosis of the bone marrow, consequence of a variety of histological changes presented in the medullary microenvironment. The development of the disease is related to the clonal expansion of myeloid stem cells, abnormal expression of cytokines, hypercellularity of myeloid lines and also extramedullary hematopoiesis. The pathophysiology of myelofibrosis involves the activation of signal transduction pathways, which may occur due to genetic rearrangements and mutations that alter the structure of protein tyrosine kinases, making hematopoietic progenitor cells independent or hypersensitive to cytokines, generating anomalous cellular behavior. Myelofibrosis is a hematological disease inserted in the group of myeloproliferative neoplasias. It has as main characteristic fibrosis of the bone marrow, consequence of a variety of histological changes presented in the medullary microenvironment. The development of the disease is related to the clonal expansion of myeloid stem cells, abnormal expression of cytokines, hypercellularity of myeloid lines and also extramedullary hematopoiesis. The pathophysiology of myelofibrosis involves the activation of signal transduction pathways, which may occur due to genetic rearrangements and mutations that alter the structure of protein tyrosine kinases, making hematopoietic progenitor cells independent or hypersensitive to cytokines, generating anomalous cellular behavior.

Aims: Recent studies have shown that microvesicles produced by the cells of the organism may be associated with the cellular communication process due to their role in the transfer and that miRNAs present in this content are able to regulate diverse cellular processes. The expression of some microRNAs is associated with hematopoietic processes such as the transformation of myeloid, erythroid and megakaryocytic progenitors. These can regulate the hematopoiesis of normal stem cells and also of compromised progenitors, having a relevant role in the pathogenesis of the disease. The development of the disease is related to the clonal expansion of myeloid stem cells, abnormal expression of cytokines, hypercellularity of myeloid lines and also extramedullary hematopoiesis. The pathophysiology of myelofibrosis involves the activation of signal transduction pathways, which may occur due to genetic rearrangements and mutations that alter the structure of protein tyrosine kinases, making hematopoietic progenitor cells independent or hypersensitive to cytokines, generating anomalous cellular behavior.

Methods: Microvesicles were isolated from plasma by ultracentrifugation method described previously. RNA was extracted using the QIAGEN kit (Qiagen, Valencia, CA, USA). Differential expression, miR-29a and miR-155 were less expressed in MF patients compared to healthy donors (P<0.02) and P <0.03), and miR-223 did not Present a statistically significant difference. Data on miR-29a corroborate in part with the literature, since the data presented here relate to miRNA carried by VEs rather than serum / plasma. However, low levels of miR-29a expression are related to abartent auto-renewal of hematopoietic progenitor cells, thus indicating that VEs may contribute to this mechanism. As for miR-155, the data obtained do not corroborate with the literature and, possibly, the VEs do not participate in a specific mechanism of regulation of Megacariopoiesis by miR-155. We used the miRNAs described in the literature as influential factors in the process of hematological disorders. They are: mir146b, mir 150, mir 29a and 155.After analysis of miRNA differential expression, miR-29a and miR-155 were less expressed in MF patients compared to healthy donors (P < 0.02 and P < 0.03), and miR-223 did not presented a statistically significant difference. Data on miR-29a corroborate in part with the literature, since the data presented...
Background: Myeloproliferative neoplasms (MPNs) are a group of chronic myeloid cancer characterized by overproduction of mature hematopoietic cells. Mutations in one of three genes; Janus kinase 2 (JAK 2), myeloproliferative leukemia virus oncogene (MPL) and calreticulin (CALR), have been identified in more than 50% of patients with CBR-Ab negative MPNs. JAK2 mutations are present virtually all cases of Polycythemia Vera and 50-60% of prMF and Essential Thrombocytosis (ET). Recently, mutations in CALR gene were found in 50-80% of JAK2 and MPL mutation negative ET and prMF patients. Aims: To evaluate immunohistochemical results of CALR gene mutation in the bone marrow samples of the JAK2V617F mutated and JAK2V617F wild type Primary Myelofibrosis (PRMF) patients. Methods: Material: Bone marrow biopsy samples from 32 patients previously diagnosed as primary myelofibrosis with known JAK V617F mutation status were obtained from archives of Marmara University Pathology Laboratory. Bone marrow samples of two patients were already known as CALR mutated by PCR analysis. Bone marrow samples of three JAK2 wild type and CALR mutated ET, two JAK2 wild type, CALR mutated prMF patients and two CALR wild type ET patients were used as positive and negative control tissues for CALR immunohistochemistry. Immunohistochemistry: 4-μm unstained sections of each bone marrow biopsy specimens were cut onto electrostatically charged glass slides. Immunohistochemistry was performed on an automated immunostainer (Ventana Benchmark Ultra; Ventana Medical Systems, Inc.). CALR antibody (clone CAL2, Dianova, Germany) staining used a 1:100 dilution. Any cytoplasmic staining of the cells with CAL2 antibody was considered positive immunostaining. Results: We studied 32 bone marrow specimens of primary myelofibrosis with 15 (47%) of them having JAK2 V617F mutation and 17 (53%) of them lacking JAK2 V617F mutation. CALR immunoactivity was seen in 8 (25%) of all pr MF patients, 2 (100%) of patients with CALR mutated ET, 2 (100%) of patients with JAK2V617F mutation. CALR immunoactivity was seen in 3 (100%) of patients with ET and 2 (100%) of patients with known CALR mutation. CALR immunoactivity was seen in 4 patients with CALR wild type ET patients. We observed that CAL2 immunostaining was seen mainly in the cytoplasm of the small and large megakaryocytes, and atypical megakaryocytes as found in fibrotic PRMF. Pale immunostaining was seen in myeloid and erytroid cell precursors. This immunostain also stained some small cells appearing as micromegakaryocytes. Summary/Conclusions: An immunohistochemical stain easily detects the CALR mutation by staining of megakaryocytes in formalin-fixed bone marrow biopsy specimens. This method would be a easy, rapid, and cost effective way to detect CALR mutations in daily routine hematopathology biopsy evaluation of the myeloproliferative patients.

PB2029

CD177 EXPRESSION IN PERIPHERAL BLOOD NEUTROPHILS IN HEALTH AND DISEASE STATES

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1Clinical and chemical pathology, 2Internal medicine, Cairo university, Cairo, Egypt

Background: Objective and specific assays are required in the identification of both chronic myeloproliferative disorders and myelodysplastic syndromes. Aims: Exploration of the possibility of using the CD177 expression in the peripheral blood neutrophils for the diagnosis of either entity. Methods: The 213 subjects were organized into 4 main groups; benign neutrophil leukocytosis group, secondary erythrocytosis group and clonal myeloid neoplasms group together with a haematologically normal group as controls. All cases were subjected to clinical assessment as well as the flow cytometry determination of the percentage (%) and mean fluorescent intensity (MFI) of peripheral blood neutrophils expressing CD177. Results: Skewed high peripheral blood neutrophil CD177 MFI was significantly associated with Philadelphia-negative cMPDs patients (2.9–37.4; median 14.1) compared to controls (0.8-20.5; median 8.8). The MDS patients did not show a significant difference in either CD177% or MFI compared to the controls. Polychthemia Vera (PV) patients had similar results of CD177 expression (% and MFI) compared to Essential Thrombocytosis (ET) patients. However, they had higher CD177 MFI levels compared to the secondary erythrocytosis patients and controls (4.8-37.4; median 16.5, 1.58-25.7; median 5.81, 0.85-20.5; median 8.8 respectively). CD177 MFI showed statistically significant higher values in ET patients compared to the haematologically normal control group (2.9-34.5; median 13.4 versus 0.85-20.5; median 8.8 respectively). No correlation between CD177 expression and JAK2 V617F allele burden could be detected either PV or MDS patients. With a 20 p.d.u cutoff, the specificity of neutrophil CD177 MFI in Philadelphia-negative cMPDs patients’ diagnosis and differentiation of PV from secondary erythrocytosis was 93% and 85% respectively. The CD177% had a low accuracy of in the diagnosis of MDS patients. The CD177 patterns observed were one positive peak and bimodal pattern in PV patients. Summary/Conclusions: The CD177 expression is highly associated with Philadelphia-negative cMPDs. It could reliably represent a useful potential marker in detecting those disorders and differentiating them from reactive cases.
LYMPHOPROLIFERATIVE DISORDER IN 3 PATIENTS ASSOCIATION OF MYELOPROLIFERATIVE NEOPLASM AND PB2031

ASSOCIATION OF MYELOPROLIFERATIVE NEOPLASM AND LYMPHOPROLIFERATIVE DISORDER IN 3 PATIENTS B. Foucher1,*, M. Dudez2, M. Daniel2, S. Girard2, C. Froelich2, F. Mestraller2, I. Tigaud3, S. Hayette4, A. Belhabri5, M.P. Pages2, L. Vila6,7

1Laboratoire, Hopital d’introduction des armées Desgenettes, 2Laboratoire, Groupement Hospitalier EST, 3laboratoire, Groupement hospitalier Sud, 4department of cytogenetics and molecular biology, Groupement Hospitalier EST, 5department of oncology and hematology, Centre Leon Barad, 6laboratoire, Groupement Hospitalier EST, 7laboratoire, Centre Léon Bérard, Lyon, France

Background: Lymphoproliferative disorders (LPD) and myeloproliferative neoplasms (MPN) are two very different sets of hematological pathologies. However, several studies have shown that the risk for LPD onset in patients with MPN is higher than in the general population (1)(2). No single LPD seems to be more at cause and all MPN are likely to present the onset of an associated LPD.

Aims: We present 3 cases diagnosed in the Department of Hematology, « Groupement Hospitalier Est », Lyon, France, of patients bearing an association of MPN and LPD: an essential thrombocytopenia (ET) with myeloma, ET with marginal zone lymphoma and a chronic myeloid leukemia with chronic lymphoid leukemia.

Methods: Diagnosis have been made thanks to cytology of peripheral blood, bone marrow aspirate and biopsy and confirmed by cytogenetic and molecular biology techniques.

Results: Case number 1. A 68 year old woman known to have essential thrombocytopenia as a MPN, with V617F mutation of the JAK2 protein kinase. After 19 years of treatment by Hydrea, she developed a splenomegaly, anaemia and slight lymphocytosis of 4.77 G/L. The blood smear, the bone marrow aspirate and biopsy examination revealed myelofibrosis evolution and an infiltration by 30% of a small sized clonal lymphoid population CD20+, CD5- Medullar karyotype was normal: 46, XX[10]. In conclusion the ET has evolved into myelofibrosis and is associated with a lymphoproliferative syndrome, possibly marginal zone lymphoma. No additional treatment has been implemented. Case number 2. A 64 year old woman know to have ET with V617F mutation of the JAK2 protein kinase treated by acetic salicylic acid. 5 years after, she presented with IgG kappa type monoclonal gammapathy up to 28 g/L, without any associated clinical manifestations nor cytopenia. Medullar blood was diluted but showed slightly atypical plasmaocytes remaining under 10%. Myeloma was diagnosed anyway and the patient received 5 cures of Velcade-Melphalan-Prednisone which resulted in complete remission. The MPN remains stable to this day. Case number 3. A 62 year old man with chronic lymphoid leukemia, treated by six cycles of R-FC. While in remission since 2 years, hemogram shows hyperleucocytosis (WBC: 18.3 G/L) with thrombocytocemia (platelets: 1866 G/L) without anemia (Hb: 13.7 g/dL). Blood smear examination reveals 3% of myeloma and basophilia (3.66 G/L). BCR-ABL transcript is positive in 43% and karyotype points out a 9;22 translocation. (46, XY, t (9 ;22) (q34 ;q11)[1] nuc ish (BLX3, BCRx3,ABL con BCRX2)[48/100].) Before starting Nilotinib, cytoreductive treatment by Hydrea was decided. Treatment is under way.

Summary/Conclusions: The three cases described highlight the diverse situations observed in cases of combined MPN/LPD pathologies. MPN with secondary onset of LPD are most frequently encountered, as was the case with patients 1 and 2. Cases of preexisting LPD and late onset MPN are rare (1), and cases of simultaneous discovery of both pathologies even more so (3). Several hypotheses have been formulated to explain the frequency of onset of these pathological associations: genomic instability due to JAK2 protein kinase activation, or due to BCR-ABL mutation, or exposure to cytotoxic chemotherapy or radiations (3).

Methods: Detection of the mutations in genes JAK2 and MPL in the diagnosis of chronic myeloproliferative disorders K. Boboev1,*, K. Karimov1, A. Mohammad1

1Institute of hematology and blood transfusion, tashkent, Uzbekistan

Background: Chronic myeloproliferative diseases is a group of clonal Ph-negative hematological diseases, which include erythremia (polycythemia Vera, PI), chronic megalakaryocytic leukemia (essential thrombocytocemia, ET) and subleukemic myelosis (primary myelofibrosis, PMF, chronic idiopathic myelofibrosis). The origin of these diseases is linked to transformation of hematopoietic stem cells, the result is the excessive production of mature cells of erythroid, granulocytic and megakaryocyte shoots with relatively long course of the disease. The frequency of occurrence of mutation V617F of gene JAK2 exon 12 and MPL gene varies in different literature.

Aims: Determination of the frequency of occurrence of mutations in genes JAK2 and MPL and identifying the importance of the verification of these diseases.

Methods: The study included 350 patients with chronic myeloproliferative diseases — with polycythemia Vera 150 patients, with essential thrombocytocemia 78, with chronic idiopathic myelofibrosis 55 and 67 patients were examined with the purpose of differential diagnosis with Ph(-) Chronic myeloproliferative diseases. The age of patients ranged from 20 to 70 years, median age was 54 years. Isolation DNA of patients was carried out using a set of reagents "AmpliPrep RIBO-prep" (OOO Interlabservice, Russia). The concentration and purity of isolated DNA was determined by Nano Drop 2000 instrument (USA). Detection of gene mutation JAK2V617F and MPL gene was carried out by standard polymerase chain reaction on a thermal cycler 2720 "Applied Biosystems" (USA), using a set of "Litech" (Moscow).

Results: The result of the research showed that the incidence of the V617F mutation in JAK2 was varying in patients depending on the type of disease. In polycythemia Vera the mutation V617F in the JAK2 gene was identified in 147 patients (98.3%), with essential thrombocytocemia in 42 patients of the 78 (54.2%), with chronic idiopathic myelofibrosis in 27 patients of 55 (49.1%). In 67 patients with no hematological profile, which examined with the purpose of differential diagnosis with Ph(-) chronic myeloproliferative diseases, V617F in JAK2 was detected in 6 (8.6%), which allowed to confirm Ph(-) Chronic myeloproliferative diseases. A mutation in exon 12 of the JAK2 gene was detected in 2 of 33 (2.9%) of those surveyed V617F JAK2-negative patients exclusively diagnosed with polycythemia Vera. The MPL V615L mutation gene was detected in polycythemia Vera and chronic idiopathic myelofibrosis 2.2% (1 of 41) and 2% (1 of 52) of patients.

Summary/Conclusions: Thus established, our data confirm that mutations in the genes JAK2 and MPL are highly specific diagnostic markers in patients with Ph-negative chronic myeloproliferative diseases.

Figure 1. Patterns of peripheral blood neutrophil CD177+ve cells expression observed in flow cytometry. A) Bimodal pattern. B, C, D, E) CD177 single positive peak varieties.

Table 1. Immunophenotypic characteristics of peripheral blood neutrophils in 3 patients.

<table>
<thead>
<tr>
<th>Case number</th>
<th>Neutrophil CD177 expression</th>
<th>CD177A</th>
<th>CD177B</th>
<th>Total CD177</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bimodal pattern</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Single positive peak</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Single positive peak</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PB2030
DETECTION OF THE MUTATIONS IN GENES JAK2 AND MPL IN THE DIAGNOSIS OF CHRONIC MYELOPROLIFERATIVE DISORDERS I. Tigaud3, S. Hayette4, A. Belhabri5, M.P. Pages2, L. Vila6,7
PB2032

CLINICAL AND ANALYTICAL DIFFERENCES BETWEEN CALR TYPE-1 AND CALR TYPE-2 MUTATION IN PATIENTS WITH ESSENTIAL THROMBOCYTHENIA AND PRIMARY MYELOFIBROSIS: A SINGLE CENTER STUDY

M. Sobas1, M. Olejniczak1, A. Andrasiak2, K. Kraszewski3, B. Jazwiec1, J. Ryba1, T. Wrobel1, K. Kulczickowski1
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Background: The JAK2V617F is a main molecular marker in myeloproliferative neoplasms (MPN) and is harbored in about 50-60% of essential thrombocythemia (ET) and primary myelofibrosis (PMF). Recently, CALR mutation was described in ET and PMF JAK2V617F negative patients. There are two main variants of CALR mutation: type 1 (a 52-bp deletion) and type 2 (a 5-bp insertion).

Aims: To compare clinical and analytical data of ET and PMF patients with CALR type-1 vs CALR type-2 mutation.

Methods: We performed a single center study on 471 patients: 87 PMF and 384 ET. The JAK2V617F mutation was analyzed in DNA from peripheral blood leukocytes by PCR ARMS method. In all JAK2V617F negative patients detection of CALR mutation was performed by fragment length analysis and the results were confirmed by sequencing. Statistical data analysis was performed using the Statistica 12.5 software for Windows.

Results: From 384 ET patients 254 were JAK2V617F positive (66%), 80 were CALR positive (21%) and 51 were JAK2V617F and CALR negative (13%). From CALR positive patients: 36 (51%) had type-1, 34 (45%) type-2 mutation, and 10 (12%) type-3 mutation. From 87 PMF patients 56 were JAK2V617F positive (64%), 18 were CALR positive (21%) and 13 (15%) were JAK2V617F and CALR negative. From CALR positive groups: 13 (72%) had type-1 and 5 (28%) had type-2 mutation. Compared with ET carrying JAK2V617F mutation, patients ET CALR positive (type-1 plus type-2) had lower hemoglobin (13.3 vs 14.5 g/dl, p<0.001) and leukocyte (8.2 vs 9.7 G/L, p<0.001), higher platelet counts (1067 vs 800 G/L, p<0.001) but with no significant differences in frequency of thrombosis. In ET, CALR mutation was associated with increased odds of myelofibrotic transformation (odds ratio [OR]=2.61; 95% CI: 1.28 - 5.34; p=0.009) comparing with JAK2V617F positive patients. Patients ET CALR type-1 had higher leukocyte counts than ET CALR type-2 mutation (9.6 vs 7.3 G/L, p= 0.008), but we did not find significant differences in hemoglobin, platelet counts, frequency of thrombosis or myelofibrotic transformation. Within PMF, no significant differences were observed. Moreover in PMF, there was no significant differences between the JAK2V617F, CALR type-1 and type-2 mutation status versus the International Prognostic Score System (IPSS).

Summary/Conclusions: We performed a single center study on JAK2 and CALR mutation was similar to previously described. Compared patients with ET JAK2V617F positive, ET CALR positive (type-1 plus type-2) had higher platelet count but no higher frequency of thrombosis was observed. Myelofibrotic transformation was more frequent in ET CALR positive versus JAK2V617F positive patients. ET patients CALR type-1 versus type-2 had higher leukocyte count but there were no more significant differences between these two groups. There were no significant differences within PMF group (to small number of patients). In PMF patients, there was no relations between IPSS and mutational status (JAK2V617F, CALR type-1 and type-2).

PB2033

ESSENTIAL THROMBOCYTHEMIA: STUDY OF TREATMENT LINES REQUIRED. EXPERIENCE OF A SINGLE CENTER

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Background: Essential thrombocythemia (ET) is a chronic myeloproliferative neoplasm that shows similar survival prognosis as general population, with a very low rate of transformation to myelofibrosis and acute leukemia. There are different treatments for these patients with optimal responses at first. For the first line, it is usually treated with hydroxyurea, although in young patients it is usually replaced by anagrelide / interferon. There are publications of hydroxyurea side effects, especially cutaneous, but there are not many studies about how many lines of treatment are needed to control the disease.

Aims: To study type and lines of treatment needed in patients with ET in a cohort of patients from January 1997 to January 2017.

Methods: We studied patients diagnosed of essential thrombocythemia in one area of the region of Murcia from January, 1997 to January, 2017. Those who started treatment and those who needed change were analyzed, either by resistance or by intolerance.

Results: In our area we have registered a total of 152 patients diagnosed with ET. Of these, 71% (108 patients) have required at least one treatment line. Table 1 shows the number of treatment lines required for the control of the disease. As is shown in the Table, more than 20% of treated patients needed a second line and 6.5% required more than 2 lines. At last, Table 3 shows current treatment of ET patients.

Table 1. Number of line treatmentes required for disease control.

<table>
<thead>
<tr>
<th>Treatment lines</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>76 (70.3)</td>
</tr>
<tr>
<td>2</td>
<td>23 (21.2)</td>
</tr>
<tr>
<td>3</td>
<td>7 (6.48)</td>
</tr>
<tr>
<td>4</td>
<td>1 (0.92)</td>
</tr>
<tr>
<td>5</td>
<td>1 (0.92)</td>
</tr>
</tbody>
</table>

PB2034

THROMBOTIC AND BLEEDING RISK FACTORS IN ESSENTIAL THROMBOCYTHEMIA

A. Zerniakova1, I. Martyntykevich1, V. Shuvaev1, L. Polushkina1, M. Fominykh1, V. Udal’eva1, I. Zotova1, D. Shichabaeva1, S. Voloshin1, B. Jazwiec1, E. Fernández-Poveda3, J.M. Moraleda-Jiménez3
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Background: Thrombosis and hemorrhage are the main category of complications, that affects the overall survival (OS), quality of life and therapy option choice in essential thrombocythemia (ET). Molecular marker presence (JAK2V617F, MPL, CALR) or its absence (triplet-negative status (TN)) in ET supposed to impact on the clinical course, thrombosis rate and ET prognosis.

Aims: The aim of this study was to investigate interactions between the presence of molecular marker, thrombosis/bleeding rates and the OS in ET. Methods: Outpatient’s charts of 240 ET patients, who had been diagnosed with ET at our institution according to WHO 2008 criteria. The following data was assessed: complete blood count, bone marrow biopsy results, bone marrow cytogenetic, the restriction fragment length polymorphism (RFLP) results used for JAK2V617F detection, in case of JAK2V617F-negative status the PCR-RFLP (MPL detection) and the direct sequencing (CALR detection) results. Different thrombotic/bleeding complications rates were analyzed. The OS in ET patients was compared according to molecular markers revealed.

Results: According to their mutational status 182/240 (75.9%) patients (pts) were JAK2V617F-positive (JAK2+), 30/240 (12.5%) – CALR-positive (CALR+), type 1 (CALR1+) – 13/30 pts (43.3%), type 2 (CALR2+) – 17/30 pts (56.7%). Only two pts were MPL-positive (MPL+) (0.8%). TN were 26/240 pts (10.8%). Among 240 pts 183 (76.3%) hadn’t any thrombotic complication or bleeding event (no complications/NC), 57/240 (23.7%) had complications: 49/57 (85.9%) reported arterial or/and venous thrombosis, stroke or heart failure (thrombosis+); 11/57 (19.3%) had bleeding events (hemorrhage+). Thrombotic complications in JAK2+ had 27.4% (50/182) pts, in TN – 30.7% (8/26) pts, in CALR1+ – 18.2% (2/11) pts and no cases of thrombosis were detected in CALR2+ and MPL+ subgroups (p<0.001). There were significant statistical differences in

Table 2. Drugs used in patients with ET.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyurea</td>
<td>99</td>
</tr>
<tr>
<td>Anagrelide</td>
<td>31</td>
</tr>
<tr>
<td>Interferon</td>
<td>10</td>
</tr>
<tr>
<td>Busulfan</td>
<td>4</td>
</tr>
<tr>
<td>Melphalan</td>
<td>1</td>
</tr>
<tr>
<td>Danazol</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 3. Current treatment of ET patients.

<table>
<thead>
<tr>
<th>Current treatment</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>34 (29 never treated, 5 no currently)</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>76</td>
</tr>
<tr>
<td>Anagrelide</td>
<td>22</td>
</tr>
<tr>
<td>Interferon</td>
<td>6</td>
</tr>
<tr>
<td>Busulfan</td>
<td>1</td>
</tr>
<tr>
<td>Danazol</td>
<td>1</td>
</tr>
<tr>
<td>Hydroxyurea + Anagrelide</td>
<td>2</td>
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</tbody>
</table>

Summary/Conclusions: This study highlights that, although ET has a very good prognosis, there is a significant percentage of patients that will need a change of treatment, either because of resistance or intolerance.
median platelet count as follows: 742x10^9/l (thrombosis+) and 937x10^9/l (hemorrhage+) (p=0.003). No significant statistical differences in median hemoglobin and leukocyte count (p=0.75 and p=0.47) were detected. There were more than half pts older than 60 years in groups NC (51%) and hemorrhage+ (59%) and in group hemorrhage+ only 36% (p<0.001). Cardiovascular risk factors were reported in 24% pts (NC), 69% pts (thrombosis+) and 36% pts (hemorrhage+) (p=0.001). There were no significant statistical differences in follows risk factors as thrombosis+ >100x10^9/l and leukocytosis >11x10^9/l (p=0.85 and p=0.72). No significant differences in OS among groups NC, thrombosis+ and hemorrhage+ (p=0.12) were found (Figure 1).

**Summary/Conclusions:** Leukocytosis >11x10^9/l and thrombocytosis >100x10^9/l cannot be assessed as independent thrombosis risk factors in ET. JAK2V617F mutation was associated with increased risk of thrombotic complications in ET. CALR mutations were correlated with lower thrombosis risk and better OS rate, comparing to JAK2+ and TN status despite the fact of CALR+ patients had higher platelets level. Along with common thrombosis risk factors (age >60 and cardiovascular risk factors) mutational status may help to identify ET course and to optimize individual therapy option choice.

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**PB2035**

DETECTION OF JAK2 EXON 12 MUTATIONS BY HETERODUPLEX ANALYSIS AND PYROSEQUENCING

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1Department of Medical Biology, Siberian Federal University, 2Krasnoyarsk branch of the Federal State budgetary institution «Hematology Research Center» Department of Health, Krasnoyarsk, 3Federal Budget Institute of Science «Central Research Institute for Epidemiology», Moscow, 4Krasnoyarsk regional hospital, 5Municipal Budget Health Service Institution «City Clinical Hospital № 7», 6Krasnoyarsk Territory Department of Health Regional state budget health facility «Krasnoyarsk interdistrict clinic №1», 7Krasnoyarsk Scientific Regional Bureau of Pathology, 8Krasnoyarsk Scientific Center of the Siberian Branch of the Russian Academy of Sciences, Krasnoyarsk, Russian Federation

**Background:** Somatic mutations in codons 533-547 of JAK2 exon 12 are highly specific to confirm the diagnosis of polycythemia vera (PV). We have previously proposed techniques for the detection and quantification of JAK2 exon 12 allele burden using a pyrosequencing method (Subbotina T et al, Haematologica, 2008). However, due to the high cost of sequencing, developing a two-stage algorithm for detect mutations in JAK2 exon 12 using inexpensive screening method is of immediate practically necessity.

**Aims:** The aim of this study was to demonstrate the feasibility of using heteroduplex analysis with separation of the PCR product by electrophoresis on non-denaturing PAGE as the preliminary screening test for detection of JAK2 exon 12 mutations.

**Methods:** 274 patients with PV or unclear erythrocytosis and with a low JAK2V617F allele burden or unmutated JAK2V617 (51 women, mean age 52.2±15.7 years and 223 men, mean age 43.6±15.6 years) were included in this study. The informed consents from these patients were obtained. The PCR with the additional stage of formation heteroduplexes was performed using the Real-time PCR kit (Syntol, Russia) and CFX 96 Real Time System (Biorad, USA). PCR products were analyzed by electrophoresis in 8% PAGE. The presence of the mutations was identified and confirmed by pyrosequencing method using PyroMark Q24 (Qiagen, Germany). To verify the presence of mutations, the DNA sequences extracted from the clinical samples were cloned into pGem-T vector using standard protocol (Promega, USA), and obtained clones were sequenced using reagents and equipment of the «Applied Biosystems» (USA). JAK2 exon 12 varianceMUT was calculated as a measure of relative changes in allele burden between the baseline and follow-up sample (Theotharides A et al, Haematologica, 2008).

**Results:** We detected JAK2 exon 12 mutation in five out 274 patients. The results of electrophoresis on non-denaturing PAGE are reported in Figure 1. The type of №1-5 patient mutations was determined by pyrosequencing: N542-E543del (c.1624_1629delAATGAA); IS450-E543delinsKK (c.1619_1627 TCA-gAAATGK (c.1622_1627delGAAATG) and p.H538_K539>L (c.1612_1616CACAATTT). These mutations have been already described. Main characteristics of 5 patients with JAK2 exon 12-mutated PV are reported in Table 1. The PV diagnosis of №1, 2, 3 and 5 patients was confirmed by bone marrow trephine biopsies histological examination. All five patients with JAK2 exon 12-mutated PV have an increased number of red blood cells, along with an accompanying increase in the concentration of hemoglobin and hematocrit level in the peripheral blood. Some of them had increase number of leukocytes and platelets in the disease dynamics. №1-4 patients was treated phlebotomy only and did not received any cytoreductive treatment to date. Patient №5 receives hydroxyurea (HU). Importantly, two out five patients with JAK2 exon 12-mutated PV also have a mutation JAK2V617 (<1%). JAK2 exon 12 allele burden in sample from №1 patient is significantly increased in the disease dynamics.

**Summary/Conclusions:** The proposed variant of the heteroduplex analysis with separation of the PCR product by electrophoresis on non-denaturing PAGE can be recommended for use as the preliminary screening test which is carried out before the confirming pyrosequencing. The two-stage approach allows to optimize the algorithm of the JAK2 exon 12 mutation detection and to improve the efficiency of testing for patients suspected of having PV in whom a JAK2V617F mutation is not detected or detected in a low allele burden. In five out 274 patients we detected JAK2 exon 12 mutation and confirmed the diagnosis of PV.

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**PB2036**

INTRODUCTION OF AN NGS GENE PANEL INTO CLINICAL SERVICE FOR MYELOPROLIFERATIVE NEOPLASMS

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**Background:** In the West Midlands region of the UK, all patients with a suspected myeloproliferative neoplasm (MPN) have access to quantitative analysis...
of JAK2 V617F by droplet digital PCR as standard of care. The British Committee for Standards in Haematology recommends that suspected MPN cases have investigation of JAK2 exon 12, CALR and MPL genes if JAK2 V617F is negative.

Aims: The aim of the project was to improve the MPN service by substituting sequential analysis of individual target regions within the JAK2, CALR and MPL genes with a single assay, and to increase the number of genes available for analysis.

Methods: A commercial next generation sequencing (NGS) gene panel (Oxford Gene Technology, SureSeq Myeloid Panel), coupled with the Illumina MiSeq platform was validated and implemented. The gene panel utilises hybridization based enrichment technology and consists of 25 MPN-related genes. During the validation stage the following were enriched and analysed: 29 positive control samples with 30 known pathogenic variants, 30 negative control samples without known pathogenic variants in the JAK2, CALR and MPL genes, and 24 MPN samples of unknown mutational status. Thus so far over 200 clinical samples have been analysed and reported since the service was introduced in October 2016.

Results: The panel has successfully identified: a large range of known pathogenic variants at high sensitivity (JAK2 V617F variant allele frequency 1%, CALR Type 1 frameshift variant allele frequency 3%), a potential alternative driver mutation in a known low level JAK2 V617F positive patient, a rare MPL exon 4 pathogenic variant and also the detection of low level CALR pathogenic variants, which would not have been detected by Sanger sequencing analysis. In one patient the panel identified the presence of two different JAK2 exon 14 pathogenic variants in cis (JAK2 V617F and JAK2 C618R). The JAK2 C618R prevented the hybridization of the probe binding site of the JAK2 V617F ddPCR assay which had led to a false negative result by ddPCR. The validation procedure also explored coverage and limits of sensitivity, potential chemistry specific artefacts and identified common polymorphisms for all 25 genes.

Summary/Conclusions: The panel has replaced the current sequential analysis of CALR, MPL and JAK2 exon 12 in JAK2 V617F negative patients and reduced turn-around-times with increased accuracy and sensitivity compared to Sanger sequencing and fragment analysis. Our current clinical service operates on a two tier system whereby clinicians can request analysis of the full 25 gene panel or a 4 gene subset (JAK2, CALR, MPL, CBL as an in silico analysis).

PB2037
IN JAK2V617F-positive myeloproliferative neoplasms, bleeding risk correlates with allele burden
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Background: Myeloproliferative neoplasms (MPN) are characterized by the presence of JAK2V617F mutation that is almost invariably associated with polycythemia vera (PV), but also occurs in the majority of patients with essential thrombocythemia (ET) or primary myelofibrosis (PMF). JAK2V617F-positive patients display different laboratory and clinical features from JAK2 wild-type, but no clear correlation was found between the JAK2V617F allele burden and natural history of the disease. The most common causes of morbidity and mortality in MPN are thrombotic and hemorrhagic complications, albeit bleedings are less frequent than thrombosis and mostly represented by minor hemor- rhagic and epistaxis, menorrhagia and gingival hemorrhage). The impact of different allele burden on bleeding risk is uncertain.
Aims: Aim of our study is to explore whether there is an association between JAK2V617F allele burden and hemorrhagic complications in a large cohort of MPN diagnosed and followed in a single center.
Methods: We selected 253 MPN (121 ET: 47.8%, 124 PV=49% and 8 PMF=3.2%) carrying JAK2V617F mutation. The median follow-up of patients was 8.8 years (0.1 – 37.3 y). Complete medical history and anti-thrombotic drugs were used recorded. Hemorrhagic complications were classified as “major” or “minor” in agreement with ISTH criteria. The patients were categorized into four quartiles based on the percentage of JAK2 mutant allele (1st quartile 1-25%, 2nd quartile 26-50%, 3rd quartile 51-75% and 4th quartile 76-100%). Nominal variables were compared with X2 test or Fisher’s exact where indicated. Survival has been evaluated only for groups with different prevalence of events during follow-up and were calculated with the Kaplan Meier method and compared using the Log Rank test.
Results: Three patients (1.2%) bled at diagnosis (1 major and 2 minor hemorrhages) while 27 (11.8%) suffered for hemorrhages during follow-up (10 major and 17 minor). Prevalence of hemorrhages results higher in 4th quartile compared both to 2nd (p=0.003) and to 1st (p<0.001) quartiles. Hemorrhages-free survival was significantly lower in 4th quartile compared both to 2nd (p=0.004) and to 1st (p<0.001). The incidence rate of hemorrhages are respectively 0.7/100 pats /y for 1st quartile, 0.65/100 pats /y for 2nd quartile, 1.26/100 pats /y for 3rd quartile and 3.23/100 pats /y for 4th quartile with a IRR of 5 and of 4.6 for the 4th quartile respectively versus 2nd and 1st one. No statistically significant differences have been noted in the use of anti-thrombotic drugs among patients of the different quartiles.
Summary/Conclusions: Risk factors for hemorrhage in MPN are not well defined, and there is no risk estimation model for this outcome. Acquired von Willebrand disease, entity of platelet increased count and aspirin use have been implicated in bleeding occurrence. Previous reports fail to demonstrate a correlation between JAK2 mutation and bleeding risk. In contrast, in our cohort we found a significantly higher incidence of bleeding manifestations during follow-up in patients with higher allele burden. Interestingly no differences were seen in administration of anti-thrombotic drugs among quartiles, suggesting an independent role of JAK2 allele burden in the different distribution of hemorrhagic events.

PB2038
JAK2 ALLELE BURDEN IN PATIENTS WITH PHILADELPHIA NEGATIVE MYELOPROLIFERATIVE NEOPLASMS
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Background: The JAK2V617F allele burden (JAK-AB) plays a central role in chronic myeloproliferative neoplasms (cMPNs); its presence has also been advocated in the differential diagnosis of cMPNs and as independent risk factor for venous thromboembolic complications. New treatment with Ruxolitinib may decrease JAK-AB but at the present, it is not clear the clinical advantage of this approach.
Aims: Primary aim of the current study was to evaluate at diagnosis the JAK-AB in patients with Philadelphia negative cMPNs, in order to evaluate any association with standard demographic, clinical and laboratory parameters with particular reference to thrombotic risk.
Methods: Peripheral blood samples from patients with Ph-negative cMPNs were collected, DNA from leucocytes was analysed for JAK-2 (V617F) gene mutation with amplification-refractory mutation system (ARMs) PCR, subsequently a real-time quantitative polymerase chain reaction (qRT-PCR) for JAK2V617F allele burden measurement was applied. A multivariate analysis was then performed to assess any association of AB with demographic and clinical data.
Results: One hundred and twelve patients with Philadelphia negative cMPNs were investigated: 52 females with a median age at diagnosis of 69 years (age range: 18-85 years), 60 males with a median age of 68 years (age range: 18-82 years). Thirty-four patients had Essential Thrombocythemia (ET), fifty-two had Polycythaemia Vera (PV) and twenty-six had primary myelofibrosis (PMF). JAK2-AB of patients with an age of <69 years and >69 years, was respectively evaluated. Patients older than 69 years showed a significantly higher JAK2-AB . JAK-AB was significantly reduced in ET, when compared to PV and PMF. No correlation was found between median values of allele burden and IPSS and DIPSS scores. In patients with PV (n=52), a significant correlation was observed between allele burden and WHO2008 scoring system. No significant correlation was found between allele burden and thrombotic risk according to IPSET-t and IPSET-ET for PV and ET, respectively. Patients with a previous history of thrombosis had the highest JAK2-AB. In PMF, a positive correlation between JAK-AB and grading of fibrosis was found only for the highest grades (PMFIII and IV). JAK-AB had a positive correlation with splenomegaly in PMF.
Summary/Conclusions: Our report cannot confirm any correlation between allele burden and thrombotic risk, according to currently adopted scoring systems. A previous history of thrombosis is however associated with the highest AB in all cases.

PB2039
COMPARISON OF CLINICAL AND LABORATORY DATA, INCLUDING JAK-2 46/1 HAPLOTYPE, BETWEEN PATIENTS WITH IDIOPATHIC ERYTHROCYTOYSIS AND POLYCYTHEMIA VERA
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Background: Idiopathic erythrocytosis (IE) is a relatively rare finding characterized by an increased red blood cell mass without an identifiable cause. Diagnosis of IE is based on the exclusion of primary and secondary erythrocytosis including JAK2-wild-type polycythaemia Vera (PV).
Aims: In the current study, we report clinical features and laboratory data able to discriminate IE from PV, at diagnosis.
Methods: We have here analyzed clinical and laboratory parameters, including JAK-2 46/1 haplotype, from patients with a confirmed diagnosis of IE and PV, followed from January 2010 to December 2015. Data were statistically analyzed, nominal variables were compared with X2 test and continuous variables with the Mann-Whitney test.
Results: Overall, 40 patients with IE and 93 patients with PV were included in the current analysis (Table 1). Splenomegaly and itch were reported only in patients with IE. History of thrombosis and cardiovascular events was positive in one case with IE. Jak-2 (V617F) and exon 12 mutations were negative in all patients with IE, while Jak-2 46/1 haplotype was found at heterozygous state in 18 patients and at homozygous state in 2 patients with IE.
PB2040

LABORATORY RESPONSIVENESS OF LOW-DOSE ASPIRIN IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

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Background: The essential thrombocythemia (ET) is a myeloid neoplasm characterized by platelet hyperreactivity and thrombosis. The daily low-dose aspirin (ASA) is a cornerstone in the prevention of the thrombotic events. In the ET an accelerated platelet turnover translates in a renewal of the drug target shortening the duration of cyclooxygenase (COX-1) inhibition and may dictate new dosing strategies particularly in ASA “low-responders” patients.

Aims: Therefore, we evaluated platelet count, β-thromboglobulin (β-TG) and platelet factor 4 (PF4), as markers of platelet activation, the platelet function activity (PFA), as indicator of ASA platelet sensitivity.

Methods: We studied 73 patients (60 patients with mean age 51 years range 32-70) with ET according to WHO criteria. The mean duration of disease was 11 years. All patients were on ASA 100 mg once daily. Of the 60 patients, 45 were on aspirin hydrochloride (daily dose 1.5 mg) (10 men, 35 women), 15 were on hydroxyurea (daily dose 2 mg) (10 men 5 women). None had inherited or acquired thrombotic risk factors. Sixty subjects served as controls. Platelets were measured by automated analyzer, β-TG and PF4 were determined by ELISA. ASA platelet sensitivity was measured by Platelet Function Analyzer (PFA-100).

Results: The mean platelet count was 455±200x10^9/L. All patients had normal β-TG and PF4 (12±5 IU/mL and 4±1 IU/mL) and prolonged C/EPI closure time (T, unit: s, n.v. 84-160 s) (249±40 s).

Summary/Conclusions: These findings suggest that IN et patients the daily low-dose ASA represents an optimal dosage strategy and that PFA test may be an useful tool to distinguish between the ASA “normal-responder” and “low-responder” ET patient.

PB2041

CLINICAL AND EXPERIMENTAL CHARACTERISTICS OF MYELOID/LYMPHOID NEOPLASMS DISPLAYING PDGFRAGRA OR PDGFRB REARRANGEMENT

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Background: According to the 2016 revision to the WHO classification of myeloid neoplasias and acute leukemia, the cases with rearrangement of tyrosine kinase (TK) genes PDGFRAGRA, PDGFRB are classified in Myeloid/lymphoid neoplasians with eosinophilia and rearrangement of PDGFR, PDGFRB, or FGFR1, or with PCM1-JAK2. It is a rare event that patients presented rearrangements with these genes. In the past decade, the dose of TKI to patients with PDGFRB and B abnormal was inconclusive.

Aims: The goal of the study was to assess the clinical and experimental characteristics and observe the response of Imatinib(IM) therapy of Myeloid/lymphoid neoplasias with PDGFRB or B abnormal.

Methods: Cytogenetic examination of bone marrow cells obtained from patients was performed by 24th culture method. R banding technique was used for karyotype analysis. PDGFRGA and B gene rearrangement were detected by FISH using triple-color of 4q12 and dual color break-apart PDGFRB probes. The fusion genes of rearrangements of PDGFRB and B genes were detected by RT-PCR. Immunophenotype analysis was carried out by flow cytometry. Most of all cases were treated with IM and followed up.

Results: The diagnoses included 27 cases of MPN, 1 case of AML-M2 and 1 case of non-hodgkin lymphoma. 21 cases were PDGFRB rearrangement, the other 8 were PDGFRB abnormal. 7 of 8 were EP fused gene, one of which concurrent with DEK-CAN fused gene, and the eight had MYO18A-PDGFRB. 7 cases of the 8 PDGFRB rearrangement had a primary abnormality with t(5;12) (q33; p13) and the other one had a secondary abnormality of AML-M2. PDGFRB and B genes rearrangement detected by FISH and multiple-RT-PCR were positive. The immunophenotypical analysis showed myeloid or lymphoid. These patients achieve rapid and durable remissions on IM.

Summary/Conclusions: In summary, patients with significantly anemia and eosinophilia should be screened for the presence of PDGFRB and B rearrangements. The dual-color FISH is a simple approach and should be added into the diagnostic work-up because these patients respond to imatinib therapy, and sustained responses have been observed. The OS of PDGFRB and B abnormal was similar with a previous report in a western population and another Chinese hematology center.

PB2042

PLATELET AGGREGATION STUDY OF ESSENTIAL THROMBOCYTHEMIA TREATED WITH ANAGRELIDE

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Background: Essential thrombocythemia (ET) is a myeloproliferative neoplasm characterized by thrombocytosis and abnormal megakaryocyte proliferation. Patients with elevated platelet count are considered to be a high-risk group for thromboembolic and/or hemorrhagic complications. In Japan, anagrelide treatment was recently approved for the 1st line as a cell reduction therapy on ET. Even now, there are few study whether the risk of thrombosis has decreased after anagrelide treatment. Moreover, the platelet count problem uncertainty remains what is the best practice to follow when the platelet count in platelet-rich plasma (PRP) exceeds about 600x10^9/L, in the recent recommendation of the standardization of light transmission aggregometry by the platelet physiology subcommittee of Scientific and Standardization Committee/International Society of Thrombosis and Hemostasis.

Aims: The aim of this study was to characterize the platelet aggregation (PA) in patients with ET. We would also clarify whether there were any changes of hemostatic side effect and platelet aggregability before and after treatment with anagrelide.

Methods: This study has been conducted with blood sample obtained from six healthy subjects, compared to 18 consecutive patients with ET. None of the patients was taking anticoagulants or cytoreductive agents. We also studied six anagrelide-treated patients with ET. Whole blood aggregometry (WBA) and LTA using PRP were performed. ADP-induced PA or collagen-induced PA used natural count PRP and platelet count adjusted PRP with platelet-poor plasma. Data were compared in the groups using the Tukey-Kramer test. This study was approved by the Ethical committee of our hospital. All study procedures were performed in accordance with the Declaration of Helsinki.

Results: The result of WBA was not obtained, because the filter was obstructed by giant platelets. In the natural PRP, even over 900x10^9/L, the platelet aggregability was markedly increased compared with the control (ADP-induced PA: p=0.023, collagen-induced PA: p=0.001), but, was not significantly different (ADP-induced PA: p=0.703, collagen-induced PA: p=0.986) in the count adjusted PRP. These results were not confirmed in cases with platelet counts of less than 600x10^9/L. There was no decrease in platelet aggregation before and after treatment with anagrelide (ADP-induced PA: p=0.3403, collagen-induced PA: p=0.514).

Summary/Conclusions: In the ET patients with platelet counts more than 900x10^9/L, the platelet aggregation by LTA with natural count PRP was remarkably accelerated and this data seemed to reflect the disease state. Although treatment with anagrelide showed cyto-reductive effect without any hemorrhagic complication in patients with ET, it did not fully reduce platelet aggregability.

PB2043

A SINGLE CENTRE EXPERIENCE OF MASTOCYTOSIS

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Background: Mastocytosis considered as a subcategory of myeloid neoplasms based on World Health Organization (WHO) 2016 classification, is characterized by expansion and accumulation of abnormal clonal mast cells in
one or more organs.KITD816V mutation and other KIT mutations play as driver mutations in the pathogenesis of disease. KITD816V mutation is positive in %80 of systemic mastocytosis patients. Recent studies show that high allele burden of KITD816V and high serum tryptase levels correlate with aggressive disease. Recently the importance of CD30 expression on neoplastic mast cells has been confirmed. CD30 is expressed aberrantly on neoplastic mast cells in patients with advanced systemic mastocytosis.

Aims: In this study we aimed to present demographic data, clinical follow-up and treatment of patients with mastocytosis and identify the impact of KIT D816V allele burden and expression of CD30 by mast cells in systemic mastocytosis.

Methods: We performed a retrospective study on 54 adult patients with mastocytosis (24 female, 30 male; mean age 44±13) who fulfilled WHO criteria between 2006 and 2016. These patients comprise cutaneous mastocytosis (CM) (n=10), indolent systemic mastocytosis (ISM) (n=30), smoldering systemic mastocytosis (SSM) (n=2), aggressive systemic mastocytosis (ASM) (n=4), systemic mastocytosis (SM) (n=3), mast cell leukemia (MCL) (n=4) and mast cell activation syndrome (MCAS) (n=1).

Results: At diagnosis, age of patients with advanced disease was higher than ISM and SSM group (p=0.001). Most frequent symptom of disease was skin lesion (urticaria pigmentosa) (%64). Skin lesions were significantly higher in patients with ISM and SSM than with advanced disease (p<0.009). But B symptoms were significantly higher in advanced disease variant (p=0.013). Anemia, trombocytopenia, elevation of ALP and GGT, hypalbuminemia were significantly higher in advanced disease than in ISM and in SSM. Osteopenia was higher in patients with ISM and SSM than with advanced disease (%56 and %18 respectively. KITD816V mutation was detectable in peripheral blood in 33 of 40 mastocytosis patients (%82) with a median Ct value 36±4. Median Ct value was significantly lower in advanced SM (Ct: 32±5) than in SM and SSM (Ct: 36±4; p=0.028) showing a significantly higher allele burden. Expression of CD30 on mast cells in bone marrow biopsies with immunohistochemistry immunostaining was detectable in 20 of 32 systemic mastocytosis patients (%62). There was no significant difference expression of CD30 on mast cell between patients with ISM (%65) (13/20) and advanced SM (%87) (7/8) (p=0.371). There was no significant correlation between elevated serum tryptase level and CD30 expression (p=0.114).

Summary/Conclusions: The definition of disease subcategories in systemic mastocytosis is important for choosing the treatment modality (cytoreduction or allogeneic stem cell transplantation vs treatment of the mediator symptoms) for the individual patient. CD30 is a diagnostic marker and also a possible therapeutic target.

PB2044
JAK2 PSEUDO-KINASE AND KINASE MUTATIONS IN THE ETIOLOGY OF THROMBOCYTOSIS
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Background: Thrombocytosis is defined as an abnormally increased number of platelets (>450×109/L) in the blood counts, whose cause can be primary or secondary. Thrombocytosis is a rare congenital disease due to germ line mutations affecting thrombopoietin signaling genes such as THPO, MPL and, more recently, JAK2.

Aims: To describe five cases of persistent thrombocytosis in young patients with JAK2 mutations.

Methods: Four children (2F: 2M), median age of 8,8 years and 1 young adult (F) 21 years-old, with sustained elevation of platelet counts. None had previous history of thrombo-hemorrhagic events. Main causes of secondary thrombocytosis were excluded, and all patients tested negative for BCR-ABL1, JAK2V617F, CALR and MPL mutations. Sanger sequencing of exons 12 to 20 of JAK2 was performed in all patients. Family studies were possible in 3 families.

Results: Median CBC values: platelets- 630±90x109/L; hemoglobin - 13,3±1,2 g/dl and leukocytes- 9,3±2,3x109/L. Four different JAK2 mutations were identified in the 5 patients (Table 1): JAK2 S591L/R867Q/T875N/T875I. The patient with the JAK2 T875N mutation had a discrete splenomegaly Familial studies allowed us to confirm that JAK2 T875N mutation in 3 adults previously characterised as essential thrombocytthemia (ET) triple negative.

Summary/Conclusions: In vitro studies performed by other authors have demonstrated that JAK2 R867Q and JAK2 S591L described in familial thrombocytosis, promote JAK-STAT activation. The germline nature of JAK2 T875N mutation (in 3 adults previously described in an acute megakaryoblastic leukemia primary cell line, was confirmed in DNA obtained from hair follicle. Two patients presented a non-described JAK2 T875I mutation. Familial studies clarified the etiology of thrombocytosis in 3 adults previously diagnosed as ET triple negative. The identification of different JAK2 germline pseudo-kinase and kinase domains mutations has settled the etiology of persistent thrombocytosis in 4 children and 1 young adult. Therefore, particularly in children, after excluding the main causes of secondary and acquired thrombocytosis, JAK2 gene sequencing should be incorporated in the differential diagnosis of this condition. The characterization of these rare forms of thrombocytosis and the follow up of these patients across generations, will improve the understanding of this entity.

Table 1.
ANALYSIS OF EMERGING MOLECULAR SIGNATURES AND ASSOCIATED CLINICAL FEATURES IN MPN

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Background: Myeloproliferative neoplasms (MPNs) are a group of clonal hematological disorders that arise from transformation of a multipotent hematopoietic stem cell which includes polycythemia vera (PV), essential thrombocytemia (ET) and primary myelofibrosis (PMF). Driver mutation’s confer growth advantage on the cancer cell and most is likely selected in the tissue microenvironment within which the neoplastic cells arise. Three-quarters of these patients carry the unique JAK2V617F mutation, JAK2 exon 12 mutations are found in 5% of patients with PV, MPL exon 10 mutations are present in about 5% ET/PMF and CALR mutations are found in 50-70% patients with ET/PMF.

Aims: In this study we investigated the prevalence of these so called carriers of MPN’s from January 2007 – January 2017 reported in our center.

Methods: We analyzed 3000 samples with suspected MPN for JAK2V617F mutation by ARMS-PCR and their allele burdens were reported by Q-PCR. We have screened a cohort of 500 patients for JAK2/MPL/CALR mutations by a sequential molecular analysis which includes PCR, RT-PCR and fragment analysis.

Results: JAK2V617F mutation is present in 50% of patients with MPN. Among 600 cases submitted for sequential molecular analysis identified 372 cases with JAK2V617F mutation, 70 cases with CALR mutation, and 6 cases with MPL mutations. Allele burden study on JAK2V617F positive patients revealed that patients with ET and PMF have the lowest allele burden, those with PV have an intermediate one and those with PMF showed the highest burden. Measurement of JAK2V617F allele burden by Q-PCR for a PMF case after allogeneic transplant (ASCT) reported that allele burden of 2.9% after 20 days of transplant and a negative result after 60 days of transplant vs 13% before ASCT. CALR mutation is found in ET and PMF cases that are mutually exclusive with JAK2V617F and MPL exon 10 mutations in ET whereas 2 cases with PMF found to be positive for JAK2V617F and CALR mutations. We found 40 cases with a 52-bp deletion, 1 case with a 14-bp deletion and 26 cases with a 5bp insertion. CALR variants reported in our cohort were 54% type 1 and 46% type 2 mutations. We found a tendency towards older age among type 2 carriers compared to type 1 carriers (median age at diagnosis: 57 years versus 52 years) or compared to non-type 2 carriers (median age at diagnosis: 57 years versus 54 years). Similarly, platelet count at diagnosis tended to be higher in the subgroup of type 2 mutation carriers than in patients with the type 1 mutation while hemoglobin levels and white blood cell count were lower compared to those with non-type 2 mutation. The mutual allele burden of JAK2V617F/CALR exon indel mutations of two PMF patients found as 10%/85% and 15%/55% respectively. In our cohort, 10% of the patients with CALR mutation had anemia, 21% had splenomegaly, and 43% had megakaryocytes at time of diagnosis. Compared with JAK2 V617F-positive ET and PMF, CALR-mutant ET and PMF are clinically correlated with lower WBC, leukocyte and hemoglobin counts, higher platelet counts, and a reduced risk of thrombosis.

Summary/Conclusions: Microarray analysis of JAK2/MPL/CALR genes as molecular marker’s for MPN’s, allows the diagnosis of 95% of patients with PV, MPL exon 10 mutations are present in about 5% ET/PMF and CALR mutations are found in 50-70% patients with ET/PMF.

Aims: To assess the impact of the type I and type II mutations of CALR on the clinical and laboratory features of ET and PMF.

Methods: A multicenter retrospective study was carried out. Samples of peripheral venous blood was obtained from 149 patients with ET (n=76) and PMF (n=73). Patients that were negative for JAK2V617F and MPL515L/K mutations were studied for CALR mutations presence as described in original paper (T.Klampf, 2013). CALR Mutations were detected in 34 patients with ET (10 - men, 24 - women) and 25 patients with PMF (13 - men, 12 - women). Statistical data processing was carried out in the program STATISTICA for Windows 6.0.

Results: The frequency of mutations CALR was comparable in patients with ET and PMF (44.7% and 35.6%). Mutations of type II is 2 times more common in ET than with the TFM. 17.1% vs 9.6% (p=0.178). Mutations of type II detected in ET, in 18 cases - in PMF, type II in 13 cases - in ET and ET - in PMF. The median of follow-up period of patients with ET with type I mutation was 36 months (3-87), with type II - 22 months (2-90). In PMF, the median of follow-up in the group with type I mutation was 46 months (3-133), type II - 77 months (4-115). Hematological parameters in patients with ET showed higher levels of WBC in patients with type I mutation (p=0.043), the level of Hb in this variant was lower (p=0.009). In PMF levels of Hb were similar in the studied groups. Type of mutations had no significant effect on the number of WBC in patients with PMF. However, PLT was higher in PMF patients with type II mutations of CALR (p=0.014). Spleen size in ET patients on the time of the diagnosis date was slightly different: in type I - 106.5mm, type II - 116.6mm (p=0.076).

The type of mutation in our study had no effect on the stratification according to the IPSET. Also there were no significant differences in assessing of the effect of therapy. Spleen size on the time of the diagnosis date in PMF patients with type I mutation were slightly larger (180.9mm vs 169.9mm). Revealed increased fibrotic changes of the bone marrow (BM) in patients with type I CALR mutations (p <0.005). CALR mutation type had no influence on the distribution of patients with PMF, depending on the risk groups on the scale of IPSS and DIPSS.

Summary/Conclusions: The effect of the type of CALR mutation on the clinical and laboratory features of the ET and PMF has found. Type of CALR mutations in our study had no effect on the number of PLT in ET, but have a value for this in index in PMF. Type I mutations in ET accompanied higher WBC level and a lower level of Hb. The published studies have not shown the influence of the type of mutation in the Hb level and the number of WBC in ET. An important observation was the detection of the type of mutation on development fibrotic changes of BM in PMF. Our data are consistent with previously published studies that showed no effect on the stratification of patients according to the scale on the IPSS.

PB2047

IMPACT OF THE TYPE OF CALR MUTATIONS ON THE CLINICAL AND LABORATORY FEATURES OF ESSENTIAL THROMBOCYTHEMIA AND PRIMARY MYELOFIBROSIS

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Background: In 2013, in the majority of JAK2V617F negative patients with essential thrombocytemia (ET) and primary myelofibrosis (PMF) have been identified mutations in the 9 exon of CALR gene. Described more than 30 different mutations, subdivided into two subtypes: deletions (type I) and insertions (type II). The are data on the phenotypic effects, depending on the version of CALR mutations. However, the prognostic significance of mutations CALR is still insufficiently clear.

Aims: To assess the impact of the type I and type II mutations of CALR on the clinical and laboratory features of ET and PMF.

Methods: A multicenter retrospective study was carried out. Samples of peripheral venous blood was obtained from 149 patients with ET (n=76) and PMF (n=73). Patients that were negative for JAK2V617F and MPL515L/K mutations were studied for CALR mutations presence as described in original paper (T.Klampf, 2013). CALR Mutations were detected in 34 patients with ET (10 - men, 24 - women) and 25 patients with PMF (13 - men, 12 - women). Statistical data processing was carried out in the program STATISTICA for Windows 6.0.

Results: The frequency of mutations CALR was comparable in patients with ET and PMF (44.7% and 35.6%). Mutations of type II is 2 times more common in ET than with the TFM. 17.1% vs 9.6% (p=0.178). Mutations of type II detected in ET, in 18 cases - in PMF, type II in 13 cases - in ET and ET - in PMF. The median of follow-up period of patients with ET with type I mutation was 36 months (3-87), with type II - 22 months (2-90). In PMF, the median of follow-up in the group with type I mutation was 46 months (3-133), type II - 77 months (4-115). Hematological parameters in patients with ET showed higher levels of WBC in patients with type I mutation (p=0.043), the level of Hb in this variant was lower (p=0.009). In PMF levels of Hb were similar in the studied groups. Type of mutations had no significant effect on the number of WBC in patients with PMF. However, PLT was higher in PMF patients with type II mutations of CALR (p=0.014). Spleen size in ET patients on the time of the diagnosis date was slightly different: in type I - 106.5mm, type II - 116.6mm (p=0.076).

The type of mutation in our study had no effect on the stratification according to the IPSET. Also there were no significant differences in assessing of the effect of therapy. Spleen size on the time of the diagnosis date in PMF patients with type I mutation were slightly larger (180.9mm vs 169.9mm). Revealed increased fibrotic changes of the bone marrow (BM) in patients with type I CALR mutations (p <0.005). CALR mutation type had no influence on the distribution of patients with PMF, depending on the risk groups on the scale of IPSS and DIPSS.

Summary/Conclusions: The effect of the type of CALR mutation on the clinical and laboratory features of the ET and PMF has found. Type of CALR mutations in our study had no effect on the number of PLT in ET, but have a value for this in index in PMF. Type I mutations in ET accompanied higher WBC level and a lower level of Hb. The published studies have not shown the influence of the type of mutation in the Hb level and the number of WBC in ET. An important observation was the detection of the type of mutation on development fibrotic changes of BM in PMF. Our data are consistent with previously published studies that showed no effect on the stratification of patients according to the scale on the IPSS.

PB2048

Abstract withdrawn.
tion in a patient with F/P MLNe who received related allogeneic transplantation from brother.

Methods: A 26-year-old male patient was presented with a 4-week history of fever, fatigue, difficulty in swallowing. Physical examination revealed generalized lymphadenopathy, splenomegaly, tonsils enlargement, leukocytosis (20x10^9/L), with marked eosinophilia (4,0x10^9/L). A bone marrow aspirate showed 2% blasts, 21% eosinophils. Histological examination of an cutaneous lymph node biopsy showed diffuse proliferation of medium-sized lymphoblasts. Immunohistochemistry and flow cytometry showed that the lymphoblastic population expressed CD2, CD7, CD4, CD99, TdT and CD1a. Polymerase chain reaction (PCR) analysis from samples of the lymph node and bone marrow failed to detect cellular receptor rearrangement. A diagnosis of T-cell lymphoblastic lymphoma (T-LBL) associated with reactive eosinophilia was rendered. The patient began standard multiagent chemotherapy in accordance with ALL-2009 protocol (ClinicalTrials.gov Identifier: NCT01199333) and achieved complete clinical remission. As he was planned to conduct autologous hematopoietic stem cell transplantation (HSCT), blood hematopoietic stem cells have been successfully harvested after stimulation of hematopoiesis. However, within 10 days after the discontinuation of G-CSF he developed leukocytosis (130x10^9/L) with 21% of eosinophils (absolute number 27,3x10^9/L) and cutibial lymphadenopathy. Histological examination of lymph node showed T-LBL relapse. Bone marrow biopsy revealed the expansion of predominantly eosinophilic cells. The study was carried out to exclude second myeloproliferative disease. Molecular and cytogenetic examinations of bone marrow failed to reveal BCR-ABL, FLT3 and NPM1, but showed CEBPA (TAD2) mutation. FISH probe revealed deletion 4q(12 (F/P rearrangement), confirmed by RT-PCR in blood and bone marrow node cells after more than 2 years of follow-up. The patient was subsequently treated with imatinib mesylate at the dose 100mg daily and showed a good clinical response. After 4 months minimal residual disease still persisted in bone marrow (RT-PCR positive for F/P and PCR for CEBPA mutation) and he received an autologous HSCT from his brother. Routine testing of chimerism at 2 months after HSCT revealed the recipient DNA less than 5% and positive probe for F/P and CEBPA. We hypothesized the germinal origin of CEBPA mutation.

Results: The same N-terminal (TAD2) CEBPA mutation was found in the patient’s skin, bone node and bone marrow, and in the patient’s brother bone marrow samples. Unfortunately, no materials from parents was available for analysis at that time.

Summary/Conclusions: Germline CEBPA mutations is very rare event and have been identified as causative gene mutations in familial AML. For the first time to our knowledge this mutation was detected in patients with PDGFRα-associated MLNe. This observation is of particular interest because it will provide novel insight about the genetic basis and the additional events responsible for the course of the disease.

PB2050 DEVELOPMENT AND DESIGN OF A RANDOMIZED CONTROLLED TRIAL USING ONLINE YOGA FOR SYMPTOM MANAGEMENT IN MYELOPROLIFERATIVE NEOPLASMS PATIENTS J. Huberty1, R. Eckert1, K. Gowin2, B. Ginos2, H. Kosiorek2, A. Dueck2, L. Larkey3, R. Mesa4, J. Wheeler5, M. Campbell-Drew5, G. Taylor-Stokes6, J. Waller6, A. Duces7, A. Dukes7, B. Hay1, T. Thomas1, M. Campbell-Drew5, G. Taylor-Stokes6, J. Waller6, A. Duces7

Patients with myeloproliferative neoplasms (MPNs) suffer from symptom burdens (i.e. fatigue, weight loss, night sweats, insomnia, sexual dysfunction, and pruritus) often not alleviated by JAK inhibition. We previously linked. Observed differences between the UK and ROSW are described in Summary/Conclusions.

Methods: Patients with myeloproliferative neoplasms (MPNs), including myelofibrosis (MF), polycythemia vera (PV), and essential thrombocythemia (ET), experience a substantial disease burden. The international MPN LANDMARK survey evaluated patient-reported impact of MPNs across 6 countries. Aims: To analyze differences in disease and symptom burden of MPN patients between the UK and the Rest of the World (ROW).

Results: A total of 699 pts (UK, n=286; ROSW, n=413) and 219 physicians were included from UK and ROW, n=163 in the survey. UK patients reported more symptoms than those in ROW (55.3 vs 38.75 respectively). A higher proportion of UK patients reported experiencing symptoms compared with ROSW (e.g. fatigue and tiredness UK - 87% MF and PV, 86% ET; ROW - 64% MF, 39% PV, 45% ET). This pattern was observed for 28 of the 31 recorded symptoms. A similar difference was seen when physicians were asked about frequency of patient-reported symptoms (e.g. fatigue and tiredness UK – 90% MF, 67% PV, 70% ; ROW – 71% MF, 55% PV, 48% ET). Patients rated symptom severity from 1 (not severe at all) to 10 (worst possible). The UK was higher than ROW for the three most common symptoms; fatigue and tiredness (mean: UK 6.73, PV 5.95, ET 5.55; ROW - 4.57, PV 4.57, ET 5.55). Patient-reported impact of disease (mean: UK 6.57, ROW 5.57). This difference was not observed when physicians were asked to rate symptom severity. A overall symptom burden was calculated as a function of all patient-reported symptoms. UK patients were disproportionately represented in the high symptom burden group (39.6% vs ROSW 16.6% ) and an average overall symptom burden score of 40.1 compared with 24.1 among ROSW patients. UK patients were also more likely to have been classified with a high risk score at diagnosis (UK 22% vs ROSW 9%). Despite the consistently greater symptom burden experienced by UK patients, little difference was observed in patient satisfaction with their treatment (UK 71% satisfied vs ROSW 73%) and treatment (UK 71% satisfied vs ROSW 73%) and treatment satisfaction (UK 87% vs ROSW 90%). UK patients were more likely to disagree with the statement ‘My doctor understands how much my condition impacts my life’ (UK 39% vs 22% ROSW). UK physicians had more MPN patients under their care than ROSW (mean patients under care in last 12 months; UK 28.5, ROSW 13.15). However, UK patients were more likely to agree with the statement ‘It is safe for me to travel’ (UK 74% vs ROSW 54%).
Summary/Conclusions: UK patients perceive a higher symptom burden than ROSW in terms of frequency and severity. While UK physicians agree with regards to frequency, they didn’t perceive a greater symptom severity in their patients compared to ROSW physicians. Patient/physician disconnect was unlikely to be the cause as satisfaction was high and similar to that in ROSW. However, UK physicians not only have more patients under their care than their ROSW colleagues, they are also more likely to feel that they don’t have enough time to discuss all symptoms. This is likely to be impacting on the ability of patients and physicians to communicate fully on symptoms and to agree on the best disease management plan.

PB2052

MPN10 SCORE AND SURVIVAL OF MOLECULARLY ANNOTATED MYELOPROLIFERATIVE NEOPLASMS PATIENTS; A FIRST REPORT ON AN EGYP TIAN COHORT

R. Abdelfattah1, F. Elrefaey1, W. Elmetenawy2

Background: The vast majority of myeloproliferative neoplasms (MPNs) patients are characterized by a molecular genetic background and by variable symptoms reflecting disease burden that may correlate with prognosis.

Aims: To study the impact of driver gene mutations (JAK2, CALR, and myeloproliferative leukemia virus oncogene (MPL)) on disease burden and correlating mutational status with symptom severity calculated by MPN10 score, degree of bone marrow (BM) fibrosis, clinical characteristics and survival in MPNs patients.

Methods: MPN Symptoms Assessment Form Total Symptom Score (MPN-SAF TSS) was assessed as mean/median of 10 items: fatigue, concentration, early satiety, inactivity, night sweats, itching, bone pains, abdominal discomfort, weight loss and fever. JAK2V617F and exon12 mutations were performed by allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) while CALR and MPL mutations were assessed by high-resolution melting (HRM).

Results: 93 MPN patients (48 males and 45 females): 18 polycythemia vera (PV), 41 essential thrombocythemia (ET), 24 primary myelofibrosis (PMF), 4 Post-ET/PV-myelofibrosis (post-ET/PV-MF) were included. Median age at diagnosis was 55 years (17-75) and was lower in ET than PV and PMF patients; 44 (19-75) vs 56 (34-70) years and 56 (20-75) years, respectively (p<0.001). JAK2 mutation was positive in 53/93 (57%); 16 (90%) PV patients, 14 (34%) ET patients, 15 (62%) PMF patients, 8 (80%) post-ET/PV-MF patients (p<0.001). CALR mutation was positive in 14/93 (15%); 10 (24%) ET patients, 4 (17%) PMF patients, zero (0%) Post-ET/PV-MF patients. MPL mutation was positive in 3/93 (3%); 2 (5%) ET patients, 1 (4%) PMF patients, zero (0%) Post-ET/PV-MF patients. 23/93 (25%) patients were triple negative; 45 (87%) patients had JAK2/CALR/MPL mutants (p<0.001). After a median follow-up period of 36 months (4-115), OS of JAK2 positive mutant patients was 100% vs 90% (p=0.015) (Figure 1).

Summary/Conclusions: MPN10 score is directly affected by JAK2 and CALR positivity and can be used as a major predictor of survival in MPN patients. Triple negative ET patients in our cohort have significantly lower MPN10 score, show lower incidence of BM fibrosis and splenomegaly which may indicate a more indolent disease course.

Figure 1.
Summary/Conclusions: The AOP2014 pen was well accepted and no major difficulties were reported. The study drug performed as expected and there were no safety concerns arising from the administration of AOP2014 using the pen device. The AOP2014 pen allows for individual dosing and a patient-convenient mode of self-administration of ropeginterferon alfa-2b at home and is expected to support adherence and compliance in the long-term treatment of PV patients.

Background: Drivers mutations JAK2, CALR and MPL are mutually exclusive in Essentials thrombocytemia (ET) and these are included in the diagnostic criteria of mieloproliferative neoplasms (MPNs). Consistent with known literature, the molecular characterisation have implications in the phenotipe disease and it might be interesting to study if these are associated with the histopathological characteristics of bone marrow biopsy.

Aims: The purpose of this work is analyse the correlations between clinical, biological and histological characteristics of bone marrow biopsy and the mutational status (JAK2, CALR, MPL).

Methods: The study included 76 patients with ET diagnosed according to WHO criteria at the Haematology Department from Hospital de Jerez from January 2005 to December 2015. We examined the prevalence, and clinical and laboratory correlations of JAK2/CALR/MPL mutations. To evaluate the histology, one pathologist with expertise in haematopathology review the bone marrow biopsies corresponding to 44 patients with ET. We included only bone marrow biopsies of at least 10 mm in length and/or minimum 8 inter-trabecular areas. The pathologist only had access to age and gender data. Mutations JAK, CALR and MPL were analysed by PCR real time and sanger sequencing.

Results: There where 55 (72%) patients JAK2, 12 (15.5%) patients CALR, one patient MPL and 9 (11.8%) patients triple-negative (TN). The main clinical and laboratory features of the patients are shown in Table 1A. As can be seen, a 75% of patients belonged a high risk group, 18 (23%) patients presented thrombotic events before diagnosis and only 4 (5.3%) during the evolution. Clinical and molecular characteristics of patients as age, sex, hemoglobin level and stratification of risk were statistically significant. (Table 1A). Thromboembolic events seemed to be more frequent in patients with JAK2 mutation, although statistical significance was not achieved. The correlation between histopathological characteristics and mutational status are shown in Table 1B. We observed differences between the presence of laxes groups of megacaryocytes according with the mutational status and there were more frequently in patients with ET according with JAK2 mutation (p= 0.01). With a median of follow up of 4 years (ranger 0.3-11 años) a total of 6 patients had died. Two patients evolved to overt, one of them to acute leukaemia and the other one to myelofibrosis at 66 and 44 months from ET diagnosis respectively.

Table 1.

<table>
<thead>
<tr>
<th>Pl. No.</th>
<th>Sample</th>
<th>Subgroup</th>
<th>Initial JAK2 V617F allele (%)</th>
<th>Follow-up JAK2 V617F allele (%)</th>
<th>Differences</th>
<th>Initial CIC</th>
<th>Follow-up CIC</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EFO</td>
<td>PV</td>
<td>55.54</td>
<td>57.01</td>
<td>0.00%</td>
<td>57.00</td>
<td>57.00</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>F08</td>
<td>PV</td>
<td>66.28</td>
<td>70.13</td>
<td>-6.1%</td>
<td>64.30</td>
<td>66.28</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M02</td>
<td>PV</td>
<td>61.95</td>
<td>61.25</td>
<td>-0.8%</td>
<td>61.95</td>
<td>61.25</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>F02</td>
<td>PV</td>
<td>46.00</td>
<td>46.95</td>
<td>-0.95</td>
<td>46.00</td>
<td>46.95</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>E08</td>
<td>PV</td>
<td>11.36</td>
<td>9.48</td>
<td>-1.5%</td>
<td>11.36</td>
<td>9.48</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>E15</td>
<td>ET</td>
<td>26.50</td>
<td>26.15</td>
<td>-0.8%</td>
<td>26.50</td>
<td>26.15</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>F01</td>
<td>ET</td>
<td>25.10</td>
<td>25.05</td>
<td>-0.05</td>
<td>25.10</td>
<td>25.05</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>FN0</td>
<td>ET</td>
<td>0.27</td>
<td>0.10</td>
<td>-0.01</td>
<td>0.27</td>
<td>0.10</td>
<td></td>
</tr>
</tbody>
</table>

* Data from the first follow-up sample.
† Data from the next follow-up sample in the same patient.

Summary/Conclusions: In our study we can confirm that there are differences between clinical and laboratory finding according with mutational status, as shown in previous studies. The most consistent finding of this study was the presence of laxes groups of megacaryocytes significantly higher in those with CALR mutations. The major limitations of this study include a small number of patients and biopsies available to analysed, this might be the mayor causes for the lack of the data demonstrating clinical and histological relevance. But our results should not be underestimated because, to our knowledge, this is the second study thus has investigated this relation.

Figure 1.
Summary/Conclusions: Quantitative analysis of JAK2 mutation using ddPCR was highly correlated with pyrosequencing and might reflect clinical treatment response.

PB2056

CLINICAL IMPACT OF JAK2 AND CARLETICULIN GENE MUTATIONS ON PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

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Background: JAK2(V617F) mutation is found in approximately 60% of patients with Essential Thrombocythaemia (ET), while 5-10% of JAK2(V617F) negative ET patients carry MPL gene mutations involving codon 515. Recently, mutations at the exon 9 of calreticulin (CALR) gene have been identified in approximately 50% of patients with ET, unmutated for JAK2 and MPL.

Aims: Primary aim of the current study was to analyze the prevalence of JAK2, MPL and CALR gene mutations in patients with ET; secondary aim was to evaluate the impact of gene mutations on clinical features of ET at diagnosis.

Methods: A cohort of consecutive patients with a diagnosis of ET followed between January 2013 and June 2016 were considered. JAK2 (V617F) gene mutation was detected by PCR testing; MPL and CALR mutations were analyzed by direct sequencing methods. Thrombotic risk score was calculated according to European Leukemia Net recommendations. Data were statistically analyzed. Results: Overall, 148 patients were included: 107 (72.30%) had JAK2(V617F) gene mutation (JAK2+), 32 (21.5%) carried a mutation at exon 9 of CALR gene (CALR+), 3 (2.0%) carried a mutation at codon 515 of MPL gene, 26 (17.58%) patients were not mutated for JAK2, CALR and MPL genes (triple negative). CALR+ subjects, compared to JAK2+ patients, had a younger age at diagnosis: median 48 year (25-92) in CALR+ patients vs 72 years (18-93, respectively). Patients with MPL mutation had a median age of 82 years while triple negative group had a median age of 59 years (23-89). The median score for thrombotic risk was 0 in CALR+ patients and 1 in JAK2+, MPL+ and triple negative patients. The distribution of International Prognostic Score for Essential Thrombocythaemia (IPSET) categories was also statistically significantly different (p=0.003) for the three groups. The percentage of high-risk patients was 0% in CALR+ group, 21 (65.6%) in JAK2+ group, and 19, 30% (5/26) in the triple negative group. The IPSET model also stratified patients with statistically significant difference (p=0.001) among the three groups: the percentage of high-risk patients was 16, 66 (2/12) in the CALR+ group, 82, 35% (88/107) in the JAK2+ group, and 33, 33(9/29) in triple negative group. CALR+ patients belonged more frequently to the low/intermediate risk group than JAK2+ patients (80% versus 17.5%, p=0.05). The incidence of thrombotic events at diagnosis of ET was 0 in the CALR+ group, 28.3% (30/107) in the JAK2+ group and 23,07% (6/26) in the triple negative group. The median overall survival was not reached in any group.

Summary/Conclusions: CALR+ patients with ET are phenotypically distinct from JAK2+ and triple negative patients. We can speculate a potential protective role of CALR mutation given the absence of thrombosis in IPSS and IPSET one high-risk patients.

PB2057

RUXOLITINIB IN MYELOFIBROSIS: A MULTICENTRE EXPERIENCE FROM THE EAST OF ENGLAND

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Background: Ruxolitinib, an oral Janus Kinase (JAK)1/JAK2 inhibitor, was approved in the EU in August 2012 for treating disease-related splenomegaly by direct sequencing methods. Thrombotic risk score was calculated according to IPSETt model. The incidence of thrombotic events at diagnosis of ET was 0 in the CALR+ group, 28.3% (30/107) in the JAK2+ group and 23.07% (6/26) in the triple negative group. The median overall survival was not reached in any group.

Summary/Conclusions: CALR+ patients with ET are phenotypically distinct from JAK2+ and triple negative patients. We can speculate a potential protective role of CALR mutation given the absence of thrombosis in IPSS and IPSET one high-risk patients.

PB2058

MONITORING OF TRANSIENT MYELOPROLIFERATIVE DISORDER AND LEUKEMIA IN DOWN’S SYNDROME: A SINGLE UNIVERSITY HOSPITAL STUDY

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Background: Children with Down syndrome (DS) have a 10- to 20-fold increased risk of developing leukemia. But some patients don’t suffer leukemia even they have significant numbers of blast cell in their peripheral blood. We can speculate a potential protective role of CALR mutation given the absence of thrombosis in IPSS and IPSET one high-risk patients.

Aims: This study gathered DS patients to find some difference between leukemia and TMD, to determine a safe treatment for the DS patient.

Methods: We collect 317 patient’s blood lab results in 433 DS patients. 102 patients has leukocytosis, and in 18 case found blast cells in their peripheral blood.

Results: 12 patients have found blast in three months of life, 11 of them finally diagnosed to TMD, and only 1 patient progress to Acute Myeloid Leukemia(AML) in 98 days of his life. Other 6 patients have blast in their blood after three months of life, and underwent chemotherapy due to hematologic malignancy. All patients with leukemia has anemia at diagnosis, which is not found in TMD patients (p=0.018). In 7 Leukemia patients, 3 was acute Lymphoblastic Leukemia(ALL) and 4 was AML. ALL patients had a blast transformation additional to trisomy 21 at their diagnostic point, which didn’t found at TMD and ALL patients, even it didn’t confirm former examination.

Summary/Conclusions: DS Patient who has blast in their peripheral blood before 3 months of life need closely follow up their Complete Blood Count and Chromosome analysis to find whether TMD progress to leukemia.

PB2059

INFECTIOUS EVENTS IN A COHORT OF PATIENTS WITH MYELOFIBROSIS UNDER TREATMENT COMPARING RUXOLITINIB WITH CONVENTIONAL THERAPY, A MONOCENTRIC EXPERIENCE OF 22 PATIENTS RETROSPECTIVELY ANALYZED

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Results: The patient group was 61.4% male, with a median age of 71 years (41-91). There were 16 (36.4%) patients with PMF, 13 (29.5%) with PPV-MF, 9 (20.5%) with PET-MF, and 6 (13.6%) with post-myeloproliferative disorder (unclassified)-MF. The indication for treatment was painful splenomegaly in 20 (45.5%) patients, constitutional symptoms in 23 (52.3%), and portal hypertension in 1 (2.3%). Ruxolitinib was first-line therapy in 10 (22.7%) patients, second-line in 24 (54.5%), and third-line or greater in 10 (22.7%). Starting doses ranged from 5mg BD in 2 (4.6%), 10mg BD in 14 (31.8%), 15mg BD in 11 (25%) and 20mg BD in 17 (38.6%) patients, with occasional dose reduction/interruption primarily due to thrombocytopenia. Fifteen (34.1%) patients were IPSS 3+, 22 (50%) IPSS 2, 6 (13.6%) IPSS 1, and 1 (2.3%) IPSS 0. Mutation analysis was available for 32 (72.7%) patients, of which 29 (90.6%) were JAK2 V617F mutated, 2 (6.3%) were JAK2 V617F/exon 12-unnmutated, and 1 (3.1%) was CALR-mutated. The median duration of treatment was 16.4 months (3-45) and median time to progression was 15.5 months (7-32). Progression-free survival (PFS) was 65.9% and overall survival (OS) 68.2%. Seven patients died from AML, 5 from progressive MF, and 2 from pneumonia. Multivariate analysis showed that ‘advancing age’ and ‘excess peripheral blasts (≥1%)’ were independent risk factors of poor outcome. HR 0.08, 95% CI 1.00-1.16; p=0.024 and HR 4.38, 95% CI 1.12-17.09; p=0.033, respectively. Clinical assessment of spleen size was available for 29 (65.9%) patients and showed a reduction in splenomegaly in 16 (55.2%), an increase in 8 (26.7%) and no change in 5 (17.2%). Weight gain occurred in 32 (72.7%) and demonstrated a strong survival advantage (HR 0.21, 95% CI 0.07-0.65; p=0.006). The most common haematologic adverse events (AEs) were cytopaenias. Forty patients (90.9%) had anaemia and 22 (50%) were transfusion-dependent, compared with 29 (65.9%) and 10 (22.7%) pre-treatment, respectively. Thirteen (29.5%) patients also received an erythropoiesis-stimulating agent. Thirty-one (70.5%) patients had received transfusions (cytopenia and/or cytopenia grade 4) compared to 11 (25%) in the control treatment. The most frequent non-haematologic AEs were minor infections, documented in 17 patients (38.6%), and included lower respiratory tract infections, candidiasis, and HSV/VZV reactivation. One patient died from Aspergillus pneumonia. Twenty-nine patients (65.9%) remain on treatment.
Background: Treatment with the Janus-activated kinase (JAK) 1 and 2 inhibitor ruxolitinib decreases constitutional symptoms and spleen size in myelofibrosis. However accumulating evidences suggest that the drug also exerts substantial immunosuppressive activity. The impressive clinical activity of ruxolitinib is predominantly mediated by its profound anti-inflammatory effects modulating dendritic cell (DC) function resulting in impaired CD4+ and CD8+ activity. Several studies have shown that Ruxolitinib affects different cytokines (IL1, IL6 and TNFa) and other immune processes and has been linked to increased incidence of opportunistic and no opportunistic infections. Herein we report our experience at our Centre.

Aims: In our retrospective study we analysed myelofibrosis patients treated with Ruxolitinib and cytoreductive treatment with Hydroxurea and supportive therapy followed in our Department from 2012 to 2016 to evaluate rate of infections developed.

Methods: We reviewed 22 patients presenting myelofibrosis (median age 72, range 60-86) describing clinical and biological features (Table 1). Our aim was description of documented infections identified with conventional treatment and with Ruxolitinib. They were 11 treated with JAK inhibitors and 11 with Hydroxurea taken orally, similar for age and clinical features.

Results: A total of 22 patients consecutively diagnosed were included in this analysis. There were 15 primary and 7 secondary myelofibrosis patients. According to the Dynamic International Prognostic Scoring System (DIPSS) 8 were low risk, 10 were intermediate risk and 4 were high. A total of 5 documented infections were identified throughout the evaluation period, 4 were grade 1 and one grade 2. They are various including oral herpes simplex reactivation, pneumonia, recurrent viral flu syndromes, esophagitis fungal and urinary infections. All of them were present in the subgroup of patients undergoing therapy with Ruxolitinib (45%) after a medium time of 8 months from beginning of therapy (range 3-10). No patients received any anti-infective prophylaxis.

Median total daily dose of ruxolitinib was 10 mg (range 5-20). All of this infections were low risk, 10 were intermediate risk and 4 were high. A total of 5 infections developed. None of patients were treated with concomitant immunosuppressive therapy with Ruxolitinib and cytoreductive treatment with Hydroxurea and supportive therapy followed in our Department from 2012 to 2016 to evaluate rate of infections developed.

Summary/Conclusions: Median total daily dose of ruxolitinib was 10 mg (range 5-20). All of this infections were low risk, 10 were intermediate risk and 4 were high. A total of 5 infections developed. None of patients were treated with concomitant immunosuppressive therapy. 3 of this patients presented renal impairment (median cratinine clearance 60-86).describing clinical and biological features (Table 1). Our aim was description of documented infections identified with conventional treatment and with Ruxolitinib. They were 11 treated with JAK inhibitors and 11 with Hydroxurea taken orally, similar for age and clinical features.

Table 1.
platelet disorders, acquired deficiency of factors V and VIII, disseminated intravascular coagulation.

**Aims:** The aim of this study is to monitor the count of erythrocytes, leukocytes and platelets, as well as hemoglobin and hematocrit values as potential risk factors for the incidence of hemorrhagic complications in patients with chronic myeloproliferative neoplasms.

**Methods:** During the three-year period we monitored the occurrence of hemorrhagic complications in 139 patients of both sexes, aged between 30 and 87 years, being diagnosed with Ph-myeloproliferative neoplasm. Patients were classified into the following groups: 1. Group with polycythemia vera (PV) (61); 2. Group with essential thrombocythemia (ET) (28); 3. Group with idiopathic myelofibrosis (IMF) (25); 4. Group with unclassified myeloproliferative neoplasms (MPNs) (25). The following possible risk factors were monitored: counts of erythrocytes, leukocytes and platelets, as well as hemoglobin and hematocrit values.

**Results:** The highest percentage of hemorrhagic complications were in the group of patients with ET and IMF (p<0.01), followed by the group with MPNs (p<0.05). In all three groups, the incidence of hemorrhagic complications in patients older than 65 years of age was higher (p<0.001). The erythrocyte count ranged from 6.45-8.89 x 10^12/L, leukocyte count 1.27-21.1 x 10^9/L and the platelet count ranged from 10.2-188.6 x 10^9/L. Hemoglobin values ranged from 176-210 g/L, and hematocrit from 0.58 to 0.83 L/L. The highest erythrocyte count, the highest hemoglobin and hematocrit values, as well as the highest leukocyte count was recorded in the group of patients with PV and MPNs (p<0.001) and the lowest in the group of patients with IMF (p<0.01). Among the groups with MPNs, PV and MPNs there was no statistically significant difference in those parameters. In the group of patients with PV and MPNs hemorrhagic complications were more frequent in percentage in patients with leukocytosis and erythrocytosis, but without statistical significance. The highest platelet count was found in the group of patients with ET and MPNs (p<0.001), and the lowest in the group of patients with IMF (p<0.01). Among the group of patients with PV and MPNs there was no statistically significant difference with regard to platelet count. Hemorrhagic complications were more frequent both in patients with platelet count below 10x10^9/L (p<0.05) and in patients with platelet count over 1000x10^9/L (p<0.01). The increase in platelet count influences the adsorption of larger von Willebrand multimers on the platelet membrane, thus having an effect on their elimination from circulation and degradation.

**Summary/Conclusions:** The platelet count can be considered a significant parameter for monitoring the risk of hemorrhagic complications in patients with myeloproliferative neoplasms, particularly with ET and IMF. Deviation from the count of leukocytes, erythrocytes, hemoglobin and hematocrit values may be considered as a potential risk factor for bleeding in patients with myeloproliferative neoplasms, but further follow-up and a larger number of subjects are needed. The age of the patient can also be considered as a risk factor for the incidence of hemorrhagic syndrome in those patients. The follow-up of patients with unclassified myeloproliferative neoplasms has been particularly important, which showed a high prevalence of hemorrhagic complications, and with the purpose of their further differentiation.

**PB2063**

**CLINICAL RELEVANCE OF JAK2V617F MUTATIONAL LOAD IN PATIENTS WITH PHILIPPLEXIA NEUTROPHILIC LEUKEMIA FROM REPUBLIC OF MACEDONIA (SINGLE-CENTER EXPERIENCE)**

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**Background:** Polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) are Philadelphia chromosome negative myeloproliferative neoplasms (MPN) characterized by the expression of an acquired activated JAK2V617F mutation. Up to date, it remains controversial how one mutation can lead to expression of three different clinical MPN phenotypes. However, several studies have shown that the JAK2V617F allele burden may correlate with specific MPN entity.

**Aims:** In order to further clarify these observations, we evaluated the JAK2 mutational status and its clinical implications in 233 JAK2V617F+ patients with different MPNs from the Republic of Macedonia.

**Methods:** We conducted a single center retrospective study which included 233 patients with JAK2V617F+MPN diagnosed according to WHO criteria, with median follow-up period of 4 years. Identification of the JAK2V617F+ mutation was analyzed with the Real Time PCR method using the Larsen protocol. Based on the mutational load patients were divided in three groups: first with <10% mutational load, second with 10-50% load and third with >50% mutational load. The correlation of the allele burden with various clinical parameters was done by independent’s tests using Statgraphics 4.3 software.

**Results:** Our study showed that median allele burden was lowest in patients with ET (22.8%), followed by PV patients (37.1%) and PMF pts (49.6%) (p<0.01). A higher mutation burden (>50% vs <10%) was associated with advanced age (67.5 vs 58.5 years and 65 vs 58 years in ET and PMF pts respectively), with higher leukocyte count (12.9 vs 9.6, 8.87 vs 8.13, 8.8 vs 12.4, and 9.8 vs 8.18 in ET, PV and PMF pts respectively), with elevated erythrocyte count (5.76 vs 4.85 and 5.59 vs 4.52 in ET and PMF pts respectively), and with higher hemoglobin level (g/dL) and platelet count 10^12/L (15.45 vs 14.35 and 1071.5 vs 860.5 in ET patients respectively) (p<0.05 for all comparisons).
**Non-Hodgkin & Hodgkin lymphoma - Biology**

**PB2064**

**PERIPHERAL BLOOD CELL STUDY FROM PATIENTS WITH FOLLICULAR LYMPHOMA AND DIFFUSE LARGE B-CELL LYMPHOMA: WHAT SHOULD WE EXPECT?**

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**Background:** Follicular lymphoma (FL) may evolve to diffuse large B-cell lymphoma (DLBCL) and interactions between neoplastic cells and immune tumour microenvironment have been involved in this process. However, the potential value of the peripheral blood study to identify FL patients at high risk of progression is less known.

**Aims:** To describe the peripheral blood findings of patients with FL and DLBCL at diagnosis, and to investigate whether a particular lymphoid distribution could be associated with aggressive disease.

**Methods:** The study (performed between September 2012 and January 2017) included 52 patients (50 female) with a median age of 70.5 years (71% ≥60 years). Patients were newly diagnosed with in situ FL (n=1), Grade 1 FL (n=12), Grade 3 FL (n=11), and DLBCL not otherwise specified (n=28). In situ FL and Grade 1 FL were grouped as low-grade FL. Most patients with FL (11/13) had low and Grade 3 FL had clinical stages III/IV.

Patients with primary or secondary immunodeficiency and those who had already received corticosteroids or chemotherapy were excluded from this study. A whole blood sample was studied at diagnosis of lymphoma and prior to the start of therapy, using multicolour flow cytometry immunophenotyping and a standard FISH protocol. A single monoclonal antibody panel including reagents against CD19, CD20, CD22, kappa, lambda, CD3, CD4, CD8, CD56 and CD45 was used, and a minimum of 300,000 events were acquired on the flow cytometer. Results were expressed as the absolute number/ml of monocytes, lymphocytes, T cells, CD4, CD8 and NK cells. Polyclonal and monoclonal B lymphocytes were also identified.

**Results:** No difference in the distribution by sex or age was found between patients with FL and DLBCL. A low cell count in at least one lymphocyte population was detected in 35/52 patients (67.3%); 100% of cases had a low number of polyclonal B cells (<100/mL). Comparison of low-grade FL, grade 3 FL and DLBCL did not show any statistically significant differences regarding monocye, CD4, CD8 and total T cells. Low-grade FL and DLBCL showed the highest number of differences, involving lymphocytes (257±2439 versus 1495±671, p=0.001), NK cells (381±312 versus 204±167, p=0.03), the CD4/CD8 ratio (1.5±2.49 versus 2.06±1.44, p=0.002), and circulating monoclonal B cells, for both percentage (15.2±23.23 versus 1.94±25, p<0.001) and absolute number (8691±1758 versus 18.75±46.47, p<0.001). Grade 3 FL and DLBCL also showed a different CD4/CD8 ratio (1.16±0.45 versus 2.06±1.44, p=0.001), with a trend toward significance regarding CD4 T cells (413±184 versus 685±457, p=0.077). Grade 3 FL had a lower number of polyclonal B cells as compared to DLBCLs (664±41 versus 105±102, p=0.048). The expression of expression of monoclonal B cells was higher in low-grade FL than in grade 3 FL, in both percentage (15.2±23.23 versus 4.58±8.48, p=0.008) and number (8691±1758 versus 43.36±69.91, p=0.002) of monoclonal B cells. The number of lymphocyte subpopulations versus normal values of lymphocyte subsets was higher in grade 3 FL than in low-grade FL (p=0.03).

**Summary/Conclusions:** The peripheral lymphocyte profile in patients with FL and DLBCL is heterogeneous, but B-lymphopenia and CD4/CD8 ratio deviations are frequent findings. Regardless of clinical stage, low-grade FL had more circulating lymphoma cells and preserved lymphocyte populations than grade 3 FL. Further studies are warranted to confirm these exploratory findings and determine their clinical implications.

**PB2065**

**POTENTIALITY OF PDPK1 AS A THERAPEUTIC TARGET MOLECULE IN MANTLE CELL LYMPHOMA**

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**Background:** Mantle cell lymphoma (MCL), is cytogenetically and molecularly characterized by chromosomal translocation t(11;14)(q13;q32) for deregulated cyclin D1 (CCND1) overexpression, and has remained as one of hard-to-treat subtypes of non-Hodgkin lymphomas (NHLs).

**Aims:** The development of novel therapeutics for MCL has been urgently needed, therefore, this study investigated the potency of PB2065 as a therapeutic target molecule in MCL cell line.

**Four MCL-derived cell lines (MINO, Jeko-1, JVM-2 and Z138 cells), three diffuse large-B-cell lymphoma (DLBCL)-derived cell lines (KPUM-M53, KPUM-UH1 and A3/KAW cells) and a Burkitt lymphoma (BL)-derived cell line (Namalwa) were utilized in this study. Patient-derived biopsied specimens were obtained with informed consent and subjected to the immunohistochemical (IHC) staining of phospho- (p-) PDPK1 Ser241. Cell proliferation was assessed by a modified MTT assay. Antibodies utilized for Western blotting was performed for evaluating protein expression levels of PDPK1, p-PDK1 Ser241, p-RSK2 Ser227, and RSK2. BX-912, a specific inhibitor for PDK1, was purchased from Selleckchem (USA). RNA interference of PDPK1 was performed by transfection with specific short hairpin RNA plasmids into MCL cell lines by means of nucleofection (Lonza, Switzerland). This study was approved by the institutional review board of our institute.

**Results:** By means of IHC examination, our study revealed that PDK1 was activated through phosphorylation in tumor cells of all 7 MCL patient-derived specimens examined, and this was also observed in all 5 MCLs examined and all 5 follicular lymphomas examined. These indicated that PDK1 is generally active in various types of B-cell lymphoid neoplasms. The in vitro treatment with BX-912 for 48 hours resulted in the dose-dependent inhibition of cell proliferation in all 4 MCL cell lines (IC50<0.9–2.5 mM), and this inhibitory effect of BX-912 was more profound in MCL cell lines compared with three DLBCL cell lines (IC50<3.7–17.0 mM) and a BL cell line (IC50>2.9 mM). In addition, the flow cytometric analysis revealed that the growth inhibition of MCL cells by PDK1 blockade with BX-912 was at least partly mediated through the induction of apoptosis. As the molecular sequelae, PDK1 blockade by BX-912 induced the dephosphorylation of RSK2/ATGK and AKT activity or CDK1 expression was unaltered by BX-912 treatment in MCL cells. By gene knockdown of PDK1 by RNA interference using three different short hairpin RNAs, we further validated that the reduction of PDK1 protein caused the inactivation of RSK2 and the growth inhibition in MCL cell lines. Finally, when combined with various agents those are utilized for the treatment of MCL such as doxorubicin, etoposide, fludarabine, bortezomib, or ABT263, BX-912 showed additive/synergistic growth inhibitory effects in MCL cell lines.

**Summary/Conclusions:** Collectively, our study suggested that PDK1/RSK2 signaling axis is the potential therapeutic target in MCL.
gested that KPUM-YY1R cells harbored the distinct gene expression patterns in MCL, a gene for p-glycoprotein (P-gp) of drug transporter membrane, MGST1, a member of glutathione S-transferase (GST) families, and argininosuccinate synthetase 1 (ASS1), a rate-limiting enzyme for arginine biosynthesis. The upregulation of MDR1 (P-gp) and MGST1 were confirmed by Western blot or RT-PCR analysis in KPUM-YY1R compared with KPUM-YY1. Importantly, the addition of Pgp inhibitor or GST inhibitors, such as ethacrynic acid, at least partly restored the sensitivity to BH in KPUM-YY1R cells, indicating the functional significance of the upregulation of MDR1 and MGST1 in the development of BH resistance in MCL. In addition, BH-resistance cells were also found to express decreased mRNA level of ASS1 whose low levels have been previously reported to play tumor suppressor roles and its loss has been associated with clinical aggressiveness in various cancers.

Summary/Conclusions: This study revealed that the multi-molecular mechanisms overplayingly underlie the development of BH resistance, therefore, the acquisition of BH resistance potentially leads multiddrug resistance in MCL cells. Our results indicate that developed KPUM-YY1R cells and KPUM-YY1 cells deserve the identification of multiplex mechanisms underlying BH activity/resistance and the future development of strategy which overcomes the treatment refractoriness in MCL.

### PB2067

**COMPARISON OF OVERALL SURVIVAL ACCORDING TO BONE MARROW ASPIRATION RESULTS IN PATIENTS WITH NON-HODGKIN’S LYMPHOMA**

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**Background:** Bone marrow (BM) biopsy with or without aspiration is usually included in the staging workup for patients with non-Hodgkin’s lymphoma (NHL). According to the National Comprehensive Cancer Network guidelines, BM biopsy is mandatory for lymphoma, but aspiration is optional. Moreover, the role of BM aspiration is controversial. Other studies have shown that BM aspiration morphologically or flow cytometry is often inconsistent with biopsy and is less likely to detect lymphoma than biopsy. There are no clear guidelines regarding results that are positive in BM aspiration and negative in biopsy.

**Aims:** The aim of this study was to establish guidelines through a comparison of the overall survival (OS) of patients with NHL using morphological method.

**Methods:** We performed a retrospective analysis of BM involvement in patients with newly diagnosed NHL in the Korea University Hospital from January 1991 to December 2016. OS was compared according to the BM groups, which were divided into three groups: the group without BM involvement in both BM aspiration and biopsy, the group with atypical lymphocytes only in BM aspiration, and the group with BM involvement in biopsy regardless of BM aspiration results. Atypical lymphocytes were identified as positive in BM aspiration if they displayed cleaved nuclei, vacuolization, and granulation including lymphoid aggregates, lymphoid proliferation of mature B-cell neoplasm, and lymphoma associated hemophagocytic lymphohistiocytosis. Reactive changes, or relative lymphocytosis were excluded. OS was assessed using the Kaplan-Meier method, and the log-rank test was used for comparison between the groups. Multivariate analysis were performed using a Cox proportional hazards model.

**Results:** In total, the data of 1,773 patients, of which 391 patients had indolent NHL and 1,382 patients had aggressive NHL, were reviewed. Of the 1,773 patients, 1,148 (64.7%) yielded negative results on both BM aspiration and biopsy, 30 (1.7%) yielded positive results with atypical lymphocytes only in BM aspiration, and 190 (10.7%) yielded positive results on biopsy. Remaining 405 patients were excluded owing to inadequate results in BM aspiration and/or biopsy. Median follow-up duration was 37.62 months (range, 0-288).

At the time of Kaplan-Meier survival analysis, OS was significantly worse for patients with BM involvement in biopsy compared with those with no BM involvement regarding both aspiration and biopsy (log-rank P<0.001, log-rank P=0.184). Patients with atypical lymphocytes only in BM aspiration also had no significant difference compared with those with BM involvement in biopsy (log-rank P=0.291; Figure 1).

Multivariate analysis was performed by adjusting survival related variables such as sex, age, lactate dehydrogenase, Ann Arbor stage, Eastern Coopera-

### PB2068

**IN VIVO IMAGING OF LUMINESCENT DIFFUSE LARGE B-CELL LYMPHOMA XENOGRAFTS COMBINED WITH MASS SPECTROMETRY IMAGING IDENTIFY SPECIFIC MOLECULAR ALTERATION DURING R-CHOP RELAPSE**

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**Background:** Diffuse large B-cell lymphoma (DLBCL) is the most common B-cell non-Hodgkin’s lymphoma (NHL) throughout the world, comprising 30–35% of all NHLs, with approximately 71,000 new cases and 19,000 deaths estimated for 2014. Currently, R-CHOP, a combination of immunotherapy (Rituximab, targeting the cell surface protein CD20 expressed by B-cell lymphoma) and chemotherapy (Cyclophosphamide, doxorubicin, vincristine and prednisone), remains the most commonly used regimens for newly diagnosed advanced DLBCLs. However, as it is a biologically aggressive disease, up to one-third of patients will ultimately become refractory to initial therapy or relapse after treatment and display poor survival outcome, underlying the urgent need for novel therapeutic approaches based upon selective molecular targets. We are combining in vivo luminescent/fluorescent DLBCL xenograft models with mass spectrometry imaging (MSI) analysis to study the tumors characteristics during R-CHOP treatment and relapse. The in vivo imaging approach allows us to precisely quantify tumor development and response to therapy, as well as to determine disease heterogeneity in patients with NHL. On the other hand, MSI technique provides information regarding analyte composition at an almost cellular level. Therefore, we can identify, localize the molecules, proteins, drugs or metabolites. 2 types of analysis are performed: i) comparison between primary untreated tumors and tumors relapsing from R-CHOP therapy. ii) study of the therapy resistant and sensitive areas of each tumor.

**Aims:** Our aim is to investigate and analyze the various chemical composition of DLBCL xenografts during tumoral development and R-CHOP treatment relapse, in order to identify yet uncharacterized targets that could become alternative targets for therapy.

**Methods:** 10 millions cells of a U2932 lymphoma cell line were xenografted into 60 athymic nude immuno-deficient mice. Tumoral growth was repeatedly quantified in a non-invasive manner based on tumors luminescent signal using the in vivo imaging system (IVIS) Lumina II. R-CHOP treatment was applied to mice after primary tumoral growth. 2 types of samples are generated: i) untreated tumors, ii) tumors relapsing from R-CHOP.

Mass spectrometry imaging is then used to analyze and compare the chemical and biological profiles of DLBCL xenografts at these stages of tumoral growth.

**Results:** In vivo imaging allows us not only to precisely assess primary tumor
development but more importantly, to monitor accurately response to R-CHOP and relapse from this therapy. The tumors at different stages of response to R-CHOP therapy are being analyzed and compared from lipodermatosclerosis and proteomics point of view. Primary analysis indicate very distinctive metabolomics and lipidomic profiles between relapsed and non-treated tumors.

**Summary/Conclusions:** Combining IVS and MSI allow us for a better understanding of the disease and the treatment effects and the possible mechanisms allowing tumor cells to escape therapy. We are currently investigating in more details these different lipodermatosclerosis, metabolomics or proteomics signatures between the different stages of DLBCL response to R-CHOP treatment in order to identify new candidates for alternative therapies.

**PB2069**

**THE PROGNOSTIC ROLE OF INDOLEAMINE 2,3-DIOXYGENASE EXPRESSION IN HODGKIN’S LYMPHOMA.**

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**Background:** Indoleamine 2,3-dioxygenase (IDO) is an inducible enzyme that catalyzes the initial and rate-limiting step in tryptophan along the kynurenine pathway. IDO is a key factor maintaining immune tolerance and expression and it correlates with poor clinical outcome in different types of cancer and hematological malignancies. It also plays a role in a lot of pathophysiological processes, such as antitumor and antimicrobial defense. IDO causes immunosuppression in the tumor microenvironment by tryptophan breakdown. Although, only several reviews have been made to evaluate IDO prognostic value and its expression value in hematological malignancies.

**Aims:** The aim of the study was to assess the impact of the IDO expression on clinical outcome in Hodgkin’s lymphoma (HL).

**Methods:** A total number of 35 patients with HL were included in the group (10 males and 25 females; median age: 17-60 years; range: 38.5 years). Early stages (I-II) and advanced stages (III-IV) were diagnosed in 48.5% (17/35) and 51.4% (18/35) of patients, respectively. B-symptoms had 37.1% (13/35) of patients at the time of diagnosis. Patients were treated with ABVD or BEACOPP (14/esc) and radiation therapy. The mRNA expression level of IDO was measured in pre-treatment tumor tissue specimens from HL patients using real-time qPCR analysis.

**Results:** For 35 patients with HL, the overall response rate after the first-line therapy was 88.6% (31/35). Progression of the disease during the therapy was observed in 11.4% of patients (4/35). Among the patients, who achieved a remission, 9 had relapses. In our study, only 20% (7/35) of HL patients were IDO-positive (IDO+), while the majority of cases in the group (80%, 28/35) were IDO-neg (IDO-). There were no significant differences in the IDO expression between histological subtypes of HL. We also did not find any association between stage of disease and IDO expression in our study. Patients with the absence of IDO expression tended to have a better response to the 1st line chemotherapy comparing to patients with positive IDO expression. The overall response rate was achieved in 71.4% (27/35) of IDO+ cases and in 92.9% (28/30) of IDO- cases. The relapse or tumor progression during the disease, was more frequently, found in HL cases with IDO+ compared IDO-expression (28.5% (2/7) versus 7.1% (2/28), respectively, p=0.05). We did not register any death of patients in IDO-group, while one patient in IDO+ group died during the follow-up period (median duration – 37 months; p<0.05). 4-year EFS rate for IDO+ HL patients was 73% compared with 60% for IDO-negative HL patients (p=0.002). The prognostic significance of IDO+ expression in clinical outcome of HL (EFS) was also confirmed by multivariate analysis (HR=2.9; 95%CI 0.8-10.1, p=0.006).

**Summary/Conclusions:** On the base of the study, our findings suggest that IDO might be a promising marker for HL prognosis as well as represents an attractive target for HL immunotherapyin patients with poor outcome.

**Aims:** We aim to analyze the impact of secondary chromosomal abnormalities on treatment outcome in pediatric Burkitt leukemia.

**Methods:** Patients with BL presenting to Children Cancer Hospital in Egypt-57357 (CCHE) from July 2007 till end of December 2015, were reviewed for karyotyping, cMYC status by FISH using break apart probes, and secondary chromosomal abnormalities. These results were correlated with survival analysis.

**Results:** Among the 877 BL patients were diagnosed and treated during the study period according to the FAB/LMB 96 protocol. Majority were males (77.3%) and above 10 years of age at presentation (42%). Associated central nervous system involvement was diagnosed in 32.9% of the patients. LDH more than 2 times the upper limit was seen in 79.5%, and 52.3% of the patients suffered from tumor lysis syndrome at presentation. Informed karyotype for 86 patients demonstrated translocation of the MYC and IG genes in 54 patients (86%) while translocation of the IGK and IGL were found in 2 (3%) and 7 (11%), respectively. Secondary chromosomal abnormalities were detected in 40 (60%) patients, with 5 or more abnormalities in 4 patients, 3 chromosomal abnormalities in 13 patients, and 2 chromosomal abnormalities in 20 patients. All common secondary common chromosomal abnormality was duplication of chromosome 1q which was found in 16 patients. Other secondary chromosomal abnormalities included structural abnormality of chromosome 14q other than MYC translocation (6 patients), chromosome 6q deletion (4 patients), chromosome 13q deletion (3 patients), marker chromosome (3 patients), loss of chromosome 17 (2 patients), isochromosome 9q (2 patients), translocation of chromosome 13, trisomy 13 and trisomy 9 in one patients each. Relapse or tumor progression on chemotherapy was seen in 16% of the whole group of patients. The 5 year OS was 57.7%, while 5 year EFS was 51.6%. When comparing incidence of relapse in relation to complex karyotype, we found that nine out of 16 (56.2%) patients having complex karyotype experienced relapse whereas relapse occurred in only 6 (12.5%) patients having non-complex karyotype (p-value= 0.005).

**Summary/Conclusions:** The frequency of secondary chromosomal abnormalities in our series is in concordance with other publications with duplication 1q being the most common, followed by deletion 6q, 13q, and 17p. Complex karyotype was significantly associated with higher incidence of relapse and poor outcome.

**PB2070**

**SECONDARY CHROMOSOMAL ABNORMALITIES AND THEIR IMPACT ON TREATMENT OUTCOME IN PEDIATRIC BURKITT LEUKEMIA.**

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**Background:** Splenic lymphomas (SLs) are rare chronic lymphoproliferative neoplasms with a very incident clinical course and a non-characteristic phenotype and karyotype. The majority of patients attending SML and SDRP patients. Diagnosis was based on standard WHO criteria. In all cases, the diagnosis was based on peripheral blood and BM findings. The baseline clinical and laboratory features as well as follow-up and outcome were recorded for every patient. Rearranged IGVH genes were amplified essentially in reactions that contained only one of the 5’ leader region primers for the indicated IgVH constant family. All PCR reactions were performed using appropriate positive and negative controls. The rearranged VH genes identified for each case seemed to represent functional rearrangements because no stop codons or crippling mutations were identified.

**Aims:** The aim of our study to determine the immunoglobulin variable heavy chain (IGHV) gene usage and somatic mutation patterns in a series of SMLZ and SDRP patients.

**Methods:** We studied 24 patients with SMLZ, 40 patients with HCL and 10 patients with SDRPL. Diagnosis was based on standard WHO criteria. When all patients, the diagnosis was based on peripheral blood and BM findings. The baseline clinical and laboratory features as well as follow-up and outcome were recorded for every patient. Rearranged IGHV genes were amplified essentially in reactions that contained only one of the 5’ leader region primers for the indicated IgVH constant family. All PCR reactions were performed using appropriate positive and negative controls. The rearranged VH genes identified for each case seemed to represent functional rearrangements because no stop codons or crippling mutations were identified.

**Results:** A comparison of the VH genes to reported germline sequences in SMLZ revealed that 10 cases (83.3%) had VH3 derived from germline sequences, whereas in 20 cases (83.33%), IgVH genes were somatically mutated. A comparison of the VH genes to reported germline sequences in SDRPL revealed that 5 cases used the VH3 family VH gene segments, 2 the VH4 family, 1 the VH5 family, and 16 the VH1 family segments. The VH1 family segments were used in 16 cases. In 4 out of 24 cases (16.67%), IGHV genes were in germine or near germine configuration, whereas in 20 cases (83.33%), IGHV genes were somatically mutated. We have shown no differences in clinical and laboratory characteristics, immunophenotype, outcome or overall survival between mutated and unmutated cases of SMLZ. A comparison of the VH genes to reported germline sequences in SDRPL revealed that five cases used the VH3 family VH genes and five the VH4 family, one case of unmutated IGHV genes. A comparison of the VH genes to reported germline sequences in SDRPL revealed that five cases used the VH3 family VH genes and five the VH4 family, one case of unmutated IGHV genes. A comparison of the VH genes to reported germline sequences in SDRPL revealed that five cases used the VH3 family VH genes and five the VH4 family, one case of unmutated IGHV genes.

**Summary/Conclusions:** Our analysis also showed the selective use of VH1 genes in SMLZ cases. All SMLZ cases with rearranged IGHV genes were represented at a lower frequency (8.33% and 25%, respectively). The present study may revealed that SMLZ and SDRPL derive from different cellular origin and may use in differential diagnosis.
CELL OF ORIGIN ASSIGNMENT USING IMMUNOHISTOCHEMISTRY IS INFLUENCED BY BCL-2 EXPRESSION IN DLBCL PATIENTS TREATED WITH CHEMIO-IMMUNOTHERAPY

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Summary/Conclusions:

Background: Diffuse Large B-cell Lymphoma (DLBCL) is a heterogenous disease with variable clinical and pathologic presentations. Using gene expression profiling or Lymph2Cx assay, DLBCL can be assigned as germinal center (GCB) or non-germinal center (Non-GCB) subtype. However such assays remain cumbersome or unavailable for routine clinical care. Immunohistochemical (IHC) algorithms, such as the one proposed by Hans et al., are easy to use but demonstrated variable concordance to gene expression profiling. Importantly, cell of origin (COO) assignment appears to influence overall survival (OS) but not progression free survival (PFS). Furthermore, anti-apoptotic BCL-2 oncogene expression confers prognostic significance in GCB DLBCL but its significance in Non-GCB is unknown.

Aims: To examine the prognostic impact of cell of origin (COO) assignment in conjunction with BCL-2 expression in a cohort of DLBCL patients.

Methods: After due IRB approval, adult patients diagnosed with DLBCL and treated at our institution between 2010 – 2015 were identified. Clinical and pathologic variables were retrospectively abstracted. IHC expression was deemed positive if >30% of staining was observed. Cell of origin analysis was determined by the Hans criteria. All patients were treated with combination chemotherapy containing rituximab. Patients who died prior to receiving therapy were excluded. Categorical and continuous variables were compared using Chi-squared and Wilcoxon tests, respectively. Time to end point analysis was computed using the method of Kaplan and Meier with log ranks. Relapse, progression or death was considered an event for PFS estimation. Analysis was computed using JMP software, version 11.

Results: A total of 122 patients were identified and analyzed. Median follow up of the cohort was 21.8 (1.47 – 107) months, during which OS was 73.5% and PFS was 59.9%. Stratified by IPI, 2-year OS was 85%, 76.3%, 72% and 49.5% for low, low-intermediate, high-intermediate and high risk patients, respectively (p=0.006). After stratifying patients to GCB and Non-GCB, baseline characteristics between the strata with regards to gender, age, stage, extranodal disease, lactate dehydrogenase (LDH), International Prognostic Index (IPI) and BCL-2 expression were not significantly different.

At 2-years, PFS was significantly higher for GCB vs Non-GCB at 72.5% vs 59.9%, respectively (p=0.008) but OS was similar at 77.8% vs 69.9% (p=0.2) (Figure 1). Interestingly, BCL-2 expression predicted OS irrespective of COO assignment. Patients with BCL-2 expression had a 2-year OS of 55.6% vs 56.2% for GCB and Non-GCB, respectively. Whereas, patients without BCL-2 expression has a superior 2-year OS at 79.9% vs 78.3% for GCB and non-GCB, respectively (p=0.02).

Summary/Conclusions: COO assignment using IHC demonstrated superior PFS for GCB over non-GCB however this was mitigated by BCL-2 expression. This raises questions regarding the currently presumed pathogenesis of the different subtypes and how to utilize the currently available targeted therapies including BCL-2 inhibitors. These observations warrant further study.

Figure 1.

PB2073

ARE DIFFERENCES BETWEEN PEDIATRIC EBV-ASSOCIATED LYMPHOMAS AND CARRIERS REGARDING LATENCY PROFILE AND MICROENVIRONMENT COMPOSITION INVOLVED IN LYMPHOMAGENESIS?

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Background: Epstein–Barr virus (EBV) infects more than 90% of the population worldwide. The virus has evolved to persist-long in B-lymphocytes of infected individuals, but disruption of this tightly regulated B-cell infection could result in EBV-associated B cell lymphomas. In Argentina, primary infection is mostly subclinical and 90% of patients are seropositive by 3 years old. However, EBV presence is statistically associated with Hodgkin lymphoma (HL) and Diffuse Large B cell lymphoma (DLBCL) in patients younger than 10 years, suggesting a relationship between low age of EBV infection and B-cell lymphoma development in children from Argentina.

Aims: Given that viral latent proteins and microenvironment composition play a key role in tumor pathogenesis or control of viral infection, our aim was to compare this scenario in pediatric EBV-associated lymphomas derived from the germinal center (GCB) and post-GC subtypes.

Methods: Formalin fixed paraffin embedded (FFPE) pediatric biopsy samples from 26 DLBCL, 55 HL and 41 tonsils from EBV carriers were analyzed. IHC for LMP1, EBNA2, CD4, CD8, Foxp3 and GrB was performed, together with EBERs in situ hybridization, and positive cells were counted in the EBV+ milieu.

Results: Latency II pattern (LMP1+ EBNA2-) was predominant in HL (100%), DLBCL (55%), as well as in EBV+ CG in pediatric carriers (90%). CD4+ cell count was highest in EBV+ CG of pediatric carriers (p=0.014, Mann Whitney test), whereas statistically higher CD4+ cells were counted at the EBV+ GC in pediatric carriers (p=0.014, Mann Whitney test). On the other hand, CD8+ cells did not exhibit statistical differences neither in EBV-associated lymphomas nor in benign conditions at the GC, and the same was observed for the germinal centers (GC) (p>0.05, Mann Whitney test). In contrast, CD4+ cell count were statistically higher exclusively at EBV+ subepithelial region in tonsils, compared to EBV- counterpart (p=0.0039, Mann Whitney test). Finally, cytotoxic activity evaluated by GrB expression displayed a trend to higher mean in EBV+ DLBCL (p=0.057, Mann Whitney test) but not in HL. Concerning EBV, pediatric carriers did not show differences in cytotoxic activity according to EBV presence at the GC (p>0.05, Mann Whitney test). In fact, GrB cytotoxic activity was prevalent only at the EBV+ subepithelial region (p=0.042, Mann Whitney test).

Summary/Conclusions: Latency II pattern prevails in both pediatric EBV-associated lymphomas and in EBV+ GC from carriers, indicating that LMP1 expression may collaborate in the lymphomagenesis process at the GC in pediatric patients from our country. Cytotoxic activity against EBV infection may be only relevant in pediatric DLBCL, and in EBV+ subepithelial regions in pediatric carriers, whereas in EBV+ HL is not increased, in contrast to previously described. CD4+ T helper cell response plays a key role at the GC region in EBV carriers, by participating directly as effectors cells, by helping to the overall immune response in the control of viral infection and restrict latency expression to type II pattern, and, ultimately, by limiting the cell outgrowth. Failure in this process may trigger malignant transformation in EBV-associated lymphomas.
real-time polymerase chain reaction (qRT-PCR) was used to confirm the results of six upregulated and two downregulated lncRNAs. Bioinformatic analysis (gene ontology analysis, pathway analysis and network analysis) was performed to predict the biological functions and potential mechanisms of the differentially expressed lncRNAs in GCB DLBCL.

Results: We demonstrated that 21,539 incRNAs were expressed in all samples analyzed, of which 1,548 incRNAs were upregulated and 2,671 IncRNAs were downregulated in GCB DLBCL cell lines (OCI-Iy1 and OCI-Iy9) (24.0-fold, P<0.05). Pathway analysis indicated that 64 pathways corresponded to upregulated transcripts, and 62 pathways corresponded to downregulated transcripts (P<0.05). In addition, an lncRNA-mRNA co-expression network was constructed to identify potential target genes related to the 3 upregulated and 2 downregulated IncRNAs.

Summary/Conclusions: Our data suggested that lncRNAs may play an important role in the pathogenesis of GCB DLBCL, and profile of lncRNAs may be used as a potential biomarker in the diagnosis of DLBCL and predicting its clinical outcome.

PB2075
FLOWS CYTOMETRY IN EVALUATION OF EXTRANODAL LYMPHOMA PRESENTING AT UNUSUAL LOCATIONS COMPARED TO NODAL LYMPHOMAS
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Background: Immunophenotyping is a fundamental step in the diagnosis of hematologic lymphoma. Flowcytometric analysis at extranodal sites presents significant diagnostic challenges due to their morphological diversity. In recent years flow cytometry (FCM) has proven useful in the evaluation of nodal and extranodal lymphoproliferative disorders on samples obtained by surgical tissue scraping along with samples for cytomorphological, histological and immunohistochemical examinations.

Methods: Flowcytometric immunophenotyping (FCI) was conducted on extranodal sites of 40 cases which included 10/40 (25%) cases out of which most common site was GIT (4 cases) followed by 5/40 (12.5%), Lymph nodes (4 cases), Tonsil (1 case), cerebrospinal fluid and ascitic fluid, was performed. Aims: The aim of our study was to evaluate the efficacy of flow cytometer for the evaluation of extranodal and nodal lymphomas on 40 patients with a clinical suspicion of hematolymphoid neoplasms. Samples for flowcytometric immunophenotyping (FCI) were obtained by fine needle aspiration (FNA) or by tissue scraping along with samples for cytomorphological, histological and immunohistochemical examinations. Samples collected in isotene were submitted for FCI on 5-color Beckman Coulter FC500, using a set of mature and immature antigens markers for lymphoid cells. Results of FCI on cytological specimens, including nodal and extranodal mass from GIT, Thyroid, Kidney, Breast, Tonsil, cerebrospinal fluid and ascitic fluid, was performed.

Results: Flowcytometric immunophenotyping conducted on extranodal sites included total 10/40 (25%) cases out of which most common site was GIT (4 cases) followed by CNS (3 cases), Kidney (1 case), Thyroid (1 case), Breast (1 case), and Tonsil (1 case). Definite diagnosis using only FCI could be obtained in 25/40 (62.5%) cases in which 6/10 (60%) cases was conducted on extranodal and 19/30 (63%) cases on nodal tissue samples. The remaining 15 cases which could not be categorized by FCI included Hodgkin lymphoma (6 cases), inadequate cellularity (5 cases), Tuberculosis (2 cases), ALCCL (1 case), Mantle cell lymphoma (1 case) and Ewing/sPNET (1 case). Combining FCI with cytological findings definite diagnosis could be found in 33/40 (82.5%) cases compared to 30/40 (75%) cases, hematolymphoid neoplasms. Flowcytometry and lncRNA expression profile was found to be 100% concordance with FCI on peripheral blood/bone marrow aspirates.

Summary/Conclusions: Flowcytometric immunophenotyping along with fine needle aspiration cytology offer a rapid, simple and minimally invasive procedure for the detection of hematolymphoid neoplastic cells in solid tissue especially extranodal sites. Flow cytometry alone may not consistently provides a definite diagnosis of lymphoma subtypes but can be very helpful in diagnosing extranodal lymphoma and nodal lymphoblastic leukemia/lymphoma.

KEYWORDS: Flow cytometry, extranodal lymphoma

PB2076
POSSIBLE ROLE OF FLOW CYTOMETRY TO CHARACTERIZE INFILTRATING CD4 CELLS IN THE MICRO ENVIRONMENT OF LYMPHOMA TISSUE SAMPLES
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Background: In our previous work (Di Gaetano et al, Ann Haematol, 2014) we analyzed by flow cytometry (FC) the rich infiltrated characterizing the microenvironment of Hodgkin lymphoma (HL), mainly comprised of CD4 T lymphocytes. We confirmed that the majority of these CD4 T expressing the activation marker CD38 (CD38+) cells in HL overlapped with the subset CD4+CD26-CD38+ to identify the non-neoplastic cellular pattern in HL. A subset connectable to regulatory T (Treg) cells, because the low expression of CD26 (DPP4) added to the presence of CD39 (NTPDase) may be responsible for the generation of adenosine, which plays a major role in T-regulated immune suppression.

Aims: We wanted to test if this subset may also characterize T infiltrating lymphocytes the lymph nodes of Non-Hodgkin’s lymphomas (NHL) and to verify the expressions of the two enzymatic markers (CD26 and CD39) in microenvironments of HL and NHL analyzed by FC.

Methods: In 2016 we analyzed by FC in lymph nodes of 6 HL and in 32 NHL (12 DLBCL, 10 FL, 5 SLL, 3 MZL, 2 MCL) the CD4 T subset testing the expression of CD26, CD38, CD39.

Results: In CD4 T HL, CD39 is expressed in 44% of the subset and the increased presence (50%) of CD4+CD26-CD38+ cells is confirmed. Compared with HL, the cells of DLBCL are not statistically (Student t test) different: CD38 (64 vs 55; p=0.39), CD26-CD38+ (50 vs 46; p=0.66), CD39 (44 vs 59; p=0.15). While HL and FL cells are significantly different: CD38 (64 vs 23; p<0.05), CD26-CD38+ (50 vs 18; p<0.05), CD39 (44 vs 23; p<0.05). The other three types of NHL, few in number, show a tendency to a significant difference compared with HL.

Summary/Conclusions: The our data show the phenotypic variations in the microenvironments of different types of lymphoma emphasizing of DLBCL the similarity with HL and the difference with FL and other NHL. They also suggest a link between a activated environment (CD38+) and a high CD39, which, in addition to a low CD26, could enhance the generation of adenosine and, therefore, an increased immune suppressive activity. The profile by FC of CD4 T infiltrating can characterize lymphomas in its environment indicating also signals and biological mechanisms representative of possible therapeutic target
the clone of B lymphocytes involved in cancer. This may support that leukemic cells may contribute to create a microenvironment and to facilitate immune escape mechanisms. FC analysis of CD26 and CD39, markers likely connected with the adenosinergic pathway, in PB can represent effective parameters to determine and characterize the Treg cell in different types of lymphoma and could serve as targets in the follow-up of HL and B-NHL.

PB2078

BCL-2 AND Ki-67 AS INDEPENDENT PREDICTORS OF POOR-RISK IPI GROUP OF PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Diffuse large B cell lymphoma (DLBCL) is heterogeneous disease in terms of clinical behaviour, morphology, phenotype and genetics. Gene expression profiling has made a distinction between two entities germinal center B-phenotype (GC), activated B-center phenotype (ABC). Use of immunohistochemical algorithms for identification of these phenotypes has been translated into clinically feasible approach defining groups as GCB, non-GCB. These algorithms do not provide complete prognostic information as the International Prognostic Index (IPI) which identifies poor- and good-risk patients. The aim of our study was to better differentiate PMLBCL and CHL of the mediastinum.

Aims: To investigate the impact of bcl-2, bcl-6, CD10, MUM1 and Ki-67 on IPI-risk stratification.

Methods: We have analyzed 50 patients with DLBCL for the expression of bcl-2, bcl-6, CD10, MUM1 and Ki-67. Patients were divided into two groups, the non-GCB, GCB group and unfavorable group 1 and unfavorable group 2, according to Hans’s algorithm and Muris’s algorithm. Clinical-pathological, biochemical, hematologic and cytogenetic data have been compared with the discordant results between CL and immunohistochemical data. The impact of the expression of bcl-2, bcl-6, CD10, MUM1 and Ki-67 on IPI-risk stratification was estimated in multiple regression analysis.

Results: Group with GCB phenotype (defined by expression of bcl-2, bcl-6, CD10, MUM1 and Ki-67) has significantly better prognosis compared with poor-risk patients identified by IPI. Multiple regression analysis identified bcl-2, bcl-6, CD10, MUM1 and Ki-67 as independent predictors of IPI risk stratification. Patients with high expression of bcl-2, bcl-6, CD10, MUM1 and Ki-67 have significantly better prognosis compared with poor-risk patients identified by IPI. The impact of the expression of bcl-2, bcl-6, CD10, MUM1 and Ki-67 on IPI-risk stratification was estimated in multiple regression analysis, and was found to be statistically significant.

Summary/Conclusions: The expression of bcl-2, bcl-6, CD10, MUM1 and Ki-67 on IPI-risk stratification was estimated in multiple regression analysis, and was found to be statistically significant. These findings provide evidence for the potential use of these variables in predicting prognosis for DLBCL patients.

PB2080

CASTLEMAN’S DISEASE: HISTOLOGICAL SUBTYPES AND MICROVESSEL DENSITY

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Background: Castleman’s disease (CD) is a rare non-clonal lymphoproliferative disorder. Most of the cases are characterized by increased vascularity in the affected tissue. The disease falls into two major histological variants: plasma cell type and hyaline vascular type. However, the correlation between microvesSEL density and the subtype of the disease has not been established yet. The aim of the study was to investigate the association between microvessel density and histological type of CD.

Methods: Twenty-eight lymph nodes from patients diagnosed with CD were used for the study. The age of the patients ranged from 24 to 65 years, 14 were male and 14 were female. Three nodes without evidence of metastasis removed for breast cancer were used as controls. The diagnosis of hyaline vascular CD was based on overall preserved immunohistochemistry with typical angio-follicular hyperplasia, circular arrangement of mantle cells around hyalinized germinal centers (“onion skin” pattern). The plasma cell type of CD was confirmed by presence of perifollicular sheets of CD138+ plasma cells. Vessels were stained with CD34 antibody. Slides were scanned by the whole slide digital Panoramic scanner. Percentage of blood vessel area (vessel density index) was calculated using Panoramic Viewer software, statistical analysis was conducted with Student’s t-test.

Results: The plasma cell variant of CD was diagnosed in 8 patients, the hyaline vascular variant – in 20 patients. In control group vessels occupied 10.1±1% of the area. In patients with plasma cell variant percentage of blood vessel area was increased to 15.1±1.4% (p<0,05). Patients with hyaline vascular CD were divided into 2 groups depending on the vessel density index. In 15 patients (75%) percentage of vessel area was 6.8±2.3%, which was somewhat lower than in patients with plasma cell variant (not statistically significant). In 5 patients (25%) with hyaline vascular CD, the percentage of vessel area was higher - 12.3±1.5% (p<0.05) and did not differ from levels in patients with plasma cell variant.

Summary/Conclusions: The highest index of vessel density in the lymph node variant with plasma cell type – in 20 patients. In hyaline vascular variant, the index was characterized by significant variability, which could reflect the heterogeneity of this type of the disease. Increased density of blood vessels in the lymphoid tissue may be considered as a possible target for angiogenesis inhibitors, especially in patients with progressive disease.

PB2081

PROGNOSTIC SIGNIFICANCE OF IMMUNOHISTOCHEMICAL MARKERS IN R-CHOP TREATED DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS

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Background: Despite its clinical, morphological and molecular heterogeneity, diffuse large B-cell lymphoma (DLBCL) is the most frequent lymphoid malig-
nancy in adults. The role of immunophenotype variability for the therapeutic outcome has long been the cornerstone for DLBCL management strategy.

**Aims:** To evaluate the immunophenotypic characteristics of DLBCL and the prognostic significance of specific biomarkers such as bcl2, bcl6, CD 10 and MUM1, in a population-based cohort of patients treated with R-CHOP.

**Methods:** We performed a retrospective assessment of all cases of DLBCL diagnosed at our institution between 2005-2013. The immunohistochemical expression patterns of all DLBCL patients were analyzed and correlated with the therapeutic response to R-CHOP regimen.

**Results:** The study included 101 patients diagnosed with DLBCL, with a median age at diagnosis of 57.1 years (19-90 years) and male/female ratio of 1.3/1. Ninety-one patients were eligible for R-CHOP treatment. The median follow-up was 41 months. Out of the 90 cases analyzed by immunohistochemistry CD 10, BCL2, BCL6 and MUM1 expression was found in 17.6%, 50.5%, 72.7% and 81.8% of cases, respectively. Negative expression for CD10, as well as positive expression of BCL2 were adverse prognostic factors for 3-years overall survival (OS) and disease free survival (DFS) (OS for bcl2: 72.3 vs 89.7, p<0.05, OS for CD10: 84.1 vs 75.1, p<0.05). BCL6 and MUM1 expressions, however, did influence neither OS nor DFS.

**Summary/Conclusions:** This study confirms the prognostic value of a multi-marker assessment which includes bcl2, bcl6, CD 10 and MUM1 expression for patients R-CHOP therapy.
Summary/Conclusions: HLH triggered by LN is diagnosed in older patients than other causes secondary HLH (46-80 vs 4-8 y/o in our center), we think this is because in our experience there are not children or Young adult in HLH due to LN group. We would like to highlight that although LN is a very common HLH trigger there are a few works describing them in the literature, that is why we would like to spread our experience. We would like to emphasize in the importance of early diagnosis. Despite being a serious disease, it is still underdiagnosed, reaching the diagnosis most of the times after seeing hemophagocytic phenomena in bone marrow biopsy. Agreeing with literature, main consulting reasons are similar to our series. Correlation between neoplastic activity and immune activation, as well as test and facts which could predict evolution should be more studied. Finally we would like to address the necessity of considering this possibility in the face of a patient with fever which does not respond to antibiotics and has not clarified citopenia, as well as the importance of conducting cheap and very profitable test such as ferritin or tryglycerides. We thusly ascertained that TMA triggered by LN is diagnosed in older patients than other causes secondary HLH (46-80 vs 4-8 y/o in our center), we think this is because in our experience there are not children or Young adult in HLH due to LN group.

PB2083
MARIH, A NATIONAL NETWORK FOR RARE IMMUNOHEMATOLOGICAL DISORDERS
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Background: Health networks focused on rare diseases were created following a call for proposals from the French Ministry of Health in the summer of 2013. Their objective is to facilitate the networks to coordinate the actions being implemented by all actors involved in treating rare diseases. Of the 23 national networks identified in 2014 in France, the network for rare immunohematological rare diseases “MaRIH” brings together national reference centres and recognized centres of expertise as well as patients’ associations involved in treating those pathologies, on behalf of scientific medical societies.

Aims: Improving care, communication and training, pushing forward research development and epidemiological surveillance.

Methods: MaRIH brings together people involved in those medical pathologies: 8 national reference centres, 5 centres of expertise, more than 50 diagnosis and/or research laboratories, 9 patients’ associations on behalf of 7 scientific societies.

Results: The main missions of this network are to improve the care, the research and to educate professionals, patients as well as to disseminate more information to the general public on those rare diseases. Improving care: Thanks to its visibility (events, leaflets, website), MaRIH should help primary care doctors to more quickly diagnose and therefore provide faster and appropriate treatment based on best practice recommendations at the national level (PND5) as well as international guidelines. The network will also be setting up new documents specific to the general and hematological rare disorders through MaRIH centres so physicians in France or in other countries can have easily an expert opinion for their patients. At the same time, improving the child-adult transition was identified by the steering committee as a top priority. Communication and training: MaRIH is involved in organizing many events in France to improve the visibility of the centres and to provide education on these rare diseases. The 1st annual conference of the network took place on June 25th 2015 and the third one is planned on June 1st 2017 in Paris. Moreover, a patient’s day meeting was organized on January 30th 2016 in Paris to inform on the update status of research on their disease as well as to help patients in daily common problems (sport, psychological, transfusion...). Pushing forward research development and epidemiological surveillance: the network has appointed a research project manager for its scientific and strategic committee to support, provide stability for and add value to research centre activities. The research project manager watch out for calls for tender, set-up of new registers and continually monitor the regulations for retrospective and prospective studies, both in France and at the international level. Furthermore, MaRIH supported successfully the application of several of its members for European reference networks (Figure 1).

Figure 1.

Summary/Conclusions: The creation of these new networks allows strengthening the links between the various actors involved in the field to improve care and answer transversal questions. In this way, MaRIH pilot concerted actions to all its members around immunohematological rare diseases by: 1- increasing the visibility of the actors on the web or during events. The MaRIH website includes all the informations of the members as well as recommendations and events (www.marih.fr), 2- communication and training. MaRIH organizes two annual events, one for patients and another one for professionals. Moreover, MaRIH sends clinical cases by email to professionals and produce an annual webcast, 3- pushing forward research development and epidemiological surveillance. Thanks to his research project manager, MaRIH facilitates the submission and the set-up of new registries or clinical studies. In the future, MaRIH will continue and further develop all these actions, in close collaboration with the French Ministry of health.

PB2084
CLINICAL FEATURES AND ETIOLOGY OF PATIENTS WITH THROMBOTIC MICROANGIOPATHIES
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Background: Thrombotic microangiopathy (TMA) is a heterogeneous group of disease that has a fatal pattern of endothelial damage. TMA can be found in association with diverse clinical conditions such as carcinoma metastasis, malignant hypertension, infections, and TTP (thrombotic thrombocytopenic purpura). TTP is a rare, life-threatening multisystem disease, characterized by microangiopathic hemolytic anemia, thrombocytopenia, fever, renal dysfunction, and neurological disorders.

Aims: The purpose of this study is to evaluate the etiology associated with TMA.

Methods: All of the six TMA patients who were newly admitted to our clinic in two months period were enrolled in this study. Effectiveness, response, adverse effects and safety of plasmapheresis were evaluated using laboratory and clinical findings. (See Table 1).

Results: First patient presented with cachexia, thrombocytopenia, and TMA. He did not respond to plasmapheresis and corticosteroid treatment. We diagnosed carcinoma metastasis and liver metastasis, respectively, through bone marrow biopsy and PET (positron emission tomography). We thusly ascertained that TMA was due to carcinoma unknown primary. The second patient presented with general neurological findings like Guillain-Barre Syndrome and paraesthesia with renal failure, thrombocytopenia, and TMA. After PLEX and corticosteroid treatment, laboratory and neurological clinical recovery were observed after one month. The third patient had chronic obstructive pulmonary disease and pneumonia in anamnesis, who presented with anaemia, thrombocytopenia, fever and pneumonia findings. We conducted PLEX therapy. On the 8th day of PLEX, the patient had ana phylaxis, we performed cardio pulmonary resuscitation. The fourth patient
presented with acute renal failure with malign hypertension. We performed hemodialysis together with PLEX treatment. Because his diagnosis was acute renal failure, malign hypertension, and TMA. The fifth patient presented with epis-taxis and sepsis. He had chronic TTP diagnosis from two years ago. We diag-nosed the patient as relapse TTP. Early treatment against infection and PLEX increased his platelet counts as early as the second day of treatment. The sixth patient was a 7-month-old boy with a fever that had been going on for five days. We treated the patient with PLEX together with the corticosteroid. Because his ADAMTS13 level was very low and he had 35% schistocytes.

Table 1.

Summary/Conclusions: We diagnosed our first patient with carcinoma unknown primary, who did not respond to PLEX and corticosteroid treatment. The results we received for that patient indicate that PLEX with corticosteroid treatment alone, remain ineffective in cancer-related TMA patients. Etiology of our second patients TMA was idiopathic. His clinical and laboratory findings improved rapidly in response to PLEX and pulse corticosteroid treatment. One viral infection induced TMA patient had anaphylactic reaction receiving his 8th PLEX. Allergic reactions should always be kept in mind when administering PLEX. One patient with TMA and malign hypertension-induced renal failure was successfully treated with PLEX, hemodialysis and antihypertensive treatment. We successfully treated our bacterial infection and sepsis-induced TTP patients with PLEX and antibiotic administration. In second TMA patient, we coupled PLEX with high dosage corticosteroid treatment even though he had an infection. For he had high schistocyte count and atypical neurological find-ings ADAMTS 13 activity only may be a guide for diagnosis of TTP, but it is unreliable for a definitive one. In conclusion, diagnosis of TTP and other TMA is difficult. Etiology, clinical features, laboratory findings should all be taken into account when diagnosing TMA. While it is established that ADAM TS13 defi-ciency is the major cause in acquired TTP, finding the etiology of other TMA is determinant for a successful treatment of the latter.

PB2085

HAEMOLYSIS AS SCREENING TEST IN LYSOSOMAL STORAGE DISEASES

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Background: Lysosomal storage disorders (LSDs) are a group of rare inherited metabolic diseases, whose clinical hallmark is organomegaly among others, due to progressive accumulation of several non-catalyzed products inside the lysosomes. This storage leads to intracellular oxidative stress status triggering oxidized metabolites production as oxysterols, which are related to apoptosis and cellular eriptosis, as well as haemolysis dysregulation.

Aims: To evaluate the link between LSDs and haemolysis and if it could be used as a screening test in LSDs.

Methods: The osmotic resistance test (ORT) was evaluated in 150 samples including controls, LSDs carriers (LSDs-C) and LSDs patients (LSDs-P). Briefly, the blood was mixed with different concentrations of sodium chloride solution (NaCl) and the haemoglobin released was quantified by spectrophotometry. The raw data was normalized using isotonic solution (0.9% NaCl). The statistical analysis (non-parametric tests and ROC curves), was computed by IBM SPSS statistics v22 software and all statistical tests will be considered and taken as bilateral significance level α=0.05.

Results: The analysis shown that haemolysis at 0.48% of NaCl allow us to sort out controls vs LSDs-C/LSDs-P (AUC=0.725) whereas no significant dif-ferences were observed between LSDs-C and LSDs-P (p-value>0.05).

Summary/Conclusions: According to our results the ORT test is an useful screening test in LSDs.

PB2086

CLINICAL SIGNIFICANCE OF ELEVATED SERUM COBALAMIN (VITAMIN B12) LEVELS

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Background: Hypercobalaminemia is a frequent but underestimated abnor-mality. Elevated serum cobalamin levels may be a sign of a wide range of dis-eases like solid neoplasms, haematological disorders like chronic myeloproliferative disorders, chronic myelogenous leukemia, promyelocytic leukemia, poly-cythemia vera, hypereosinophilic syndrome as well as liver and kidney dis-eases.

Aims: We aimed to evaluate the underlying disorders of the patients with high cobalamin levels (>1000 pmol/l) between 01.02.2016-01.02.2017 in Hacette-pe University Pediatric Hematology Department.

Methods: We investigated the patient records of the patients examined between 01.02.2016-01.02.2017 in our department and included the patients with serum cobalamin levels higher than 1000 pmol/l. We excluded the patients who are taking Vitamin B12 supplement.

Results: There were 46 patients with serum cobalamin levels higher than 1000 pmol/l out of 14367 patients seen between 01.02.2016-01.02.2017 in our department. The reason to check the cobalamin levels were anemia, neu-tropenia and thrombocytopenia in most of the patients. Only 2 patients were referred to our department because of hypercobalaminemia. The underlying disorders were found to be leukemia in 3 patient (Acute lymphoblastic leukemia (ALL) n:1, acute myeloblastic leukemia (AML) n:1), large granulocytic lymphocytic leukemia (LGLL) n:1), myelodysplastic syndrome (MDS) in 2 patients, isolated thrombocytopenia in 4 patients, isolated neutropenia in 7 patients, bicytopenia in 4 patients, aplastic anemia in 2 patients, cobalamin metabolism defects in 10 patients, hypereosinophilia in 2 patients, polisitemia in 1 patient, cystic fibrosis in 1 patient, HIV in 1 patient, FMF (familial mediterrenian fever) in 1 patient, chronic kidney failure in 2 patients, sickle cell anemia in 1 patient, factor V Leiden in 1 patient, and hemochromatosis in 1 patient.

Summary/Conclusions: An observed elevation of cobalamin merits the a full diagnostic work up to assess the presence of an early diagnostic marker of these diseases. When we look at the patients except hematological neoplasm and cytopenias, most of the underlying reasons is associated with inflammation and infection, cobalamin was found to be elevated as an acute fase reactant. A certain approach is needed whether to determine the potential indications to search for high serum cobalamin levels and to determine the practical clinical strategy when elevated cobalamin levels discovered.

PB2087

THE HEMATOLOGIC FINDINGS OF INHERITED METABOLIC DISEASE; THEY ARE MORE THAN EXPECTED

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Background: Inherited metabolic diseases are pathological conditions that generally develop as a result of impairment of the production or breakdown of protein, carbohydrate and fatty acids. Hematological problems are some of the most frequently observed findings of inherited metabolic diseases. These may be seen together with other systemic findings or sometimes as the first and only diagnostic finding of disease. Early determination of hematological findings has a positive effect on the prognosis of metabolic diseases.

Aims: The aim of this study is to evaluate the incidence of hematological find-ings in inherited metabolic diseases since there are a few studies about the true incidence in literature.

Methods: Three hundred eighteen patients who were being followed-up within the previous 6 months at Gazi University Department of Pediatric Nutrition and Metabolism, Turkey, were included in the study. Patients’ hematological findings were taken from Department of Pediatric Nutrition and Metabolism and hospital data-processing records. Since patients were in different age groups, hematolog-i cal findings were compared with normal values for each patient’s age group. The hematological findings were classified under seven main groups; anemia of chronic disease, iron deficiency anemia, vitamin B12 deficiency anemia, homocystinuria, leukocytosis and thrombocytosis. Metabolic diseases were classified according to the textbook of Inborn Metabolic Diseases: Diagnosis and Treatment.

Results: Nine hundred twenty-two hematological examinations of the 318 patients were included to the study, and 282 hematological findings were deter-mined, 127 anemia of chronic disease, 80 iron deficiency anemia, 56 cytopenia and four vitamin B12 deficiency anemia. Leukocytosis (n=1), thrombocytosis (n=5) and homocystinuria (n=9) were also observed.

Summary/Conclusions: It was determined that although anemia of chronic disease and nutritional anemia are the most common hematological findings, these may be diagnosed late, while neutropenia, thrombocytopenia, pancy-topenia and homocystinuria may be diagnosed earlier. METABOLIC dis-eases must be considered in the evaluation of cytopenias, particularly in cases with an atypical cause that are resistant to treatment and have additional accompanying findings. Our study is the most comprehensive one in the liter-
PB2088
HEMATOTOXIC EFFECTS OF GENERIC TRIAZOLE FUNGICIDES
TEBUCONAZOLE ON WISTAR HANNOVER RATS
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Background: Pesticides are extensively used in agriculture today. Fungicides based on derivatives of triazole are the most widespread all over the world. Tebuconazole (TB) is one of the most frequently used substance of this group.

Literature review confirms that triazole fungicides have the ability to cause different hematotoxic effects.

Aims: Since 2007-2016 years we have investigated 10 test-substances of generic tebuconazoles (purity up to 97%) from different manufacturers with purpose to assess their hematotoxic action on males Wistar Han rats peripheral blood in the subchronic 90-days oral toxicity study (according to SOP and OECD 408 recommendations in compliance with GLP).

Methods: The Wistar Han males were randomly allotted to four groups. The input controls of peripheral blood parameters were conducted after a period of animals acclimatization. The goal was to evaluate the physiological state of the Wistar Han rats and the blood picture before treatment. Doses of 0; 10; 50; 200 mg/kg/bw/day were defined and were the same in all studies. Blood samples for hematological measurements were examined at 4, 9, 13 weeks after TB exposure in the same groups of animals throughout the experiment. Hemoglobin (HGB) concentration, hematocrit (HCT), total amount of erythrocytes (RET), white blood cells (WBC) and platelets (PLT), mean corpuscular hemoglobin (MCH) were evaluated.

Results: As a result, all generic TBs on high toxic doses level (200 mg/kg/bw/day) had shown the tendencies for quantitative hematological changes. TBs mainly provoked the significant decrease of HGB concentration and RBC count on 4th and 9th weeks of exposure. Morphological changes of RBC (anisocytosis) were seen too. It means that generic TBs had anemic effect. In general, changes of hematological parameters were not principally significant and did not differ from control values at 13th weeks of experiments, except two TB’s, which had shown significant decrease of HGB. Also some of generic TBs provoked leukopenia (leukocytes count) or leukocytosis of leukocytes count in peripheral blood. In case of generic pesticides, the presence of impurities can demonstrate various hematotoxic action. Also the adverse effects on peripheral blood of males Wistar Han rats were observed at a dose of 50 mg/kg/bw/day and demonstrated the lesions of red blood. But abovementioned changes were not so clearly expressed. Any adverse hematotoxic effects at 10 mg/kg/bw/day dose were not observed in all studies.

Summary/Conclusions: As a conclusion, due to our results the triazole fungicides generic tebuconazoles have hematotoxic action. They induce anemia in Wistar Han rats and quantitative white blood cells changes. Today it is very important to investigate the hazardous effects of pesticides on the blood system.

PB2089
WHAT WE CAN DO TO MAKE A STANDARDIZATION AND HARMONIZATION OF APTT?
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Background: Transfusion induced many kind of complication in patients therefore per-operational coagulation monitoring was suggested before the surgery to prevent the patients against unpredictable bleeding. Also there are some diseases which had bleeding events in surgical procedure or spontaneously. We should detect these kind of diseases and we should examine the correct measure of active parsel thromboplastin time (APTT) before surgical procedure by detecting the mild ormoderate deficiencies of plasma factor levels and by eliminating the lupus anticoagulant from plasma. This was caused to make the importance of APTT reagents.

Aims: We tried to show the importance of APTT reagents and how to reach the correct measure of APTT in this study.

Methods: We are planning to examine 300 patients, 109 of 300 patients were included as study patients. APTT levels were calculated ACH-TOX analyzer by using three different reagents.First reagent was Hemosil APTT-SP which was sensitive against only lupus anticoagulant. It contains mix colloidal silica and normal range of APTT-SP was 25.4-36.9 s. Second reagent was Hemosil SynthA Fox-SF which was sensitive against only lupus anticoagulant. It contains ellagic acid and normal range of APTT- SF was 21.5-30.4 s. APTT was measured by using Hemosil SynthA-SS reagent and had normal level of Hemosil SynthA-SS reagent and had normal level of APTT with Hemosil APTT-SP. Seven of 109 patients had long level of APTT by measuring Hemosil synhAsil-SS reagent and had normal level with Hemosil SynthA-SS reagent and Hemosil APTT-SP. Seventeen of 109 patients had long level of APTT by measuring Hemosil synhAsil-SS reagent and Hemosil SynthA-SS reagent and had normal level of APTT with Hemosil SynthA-SS reagent and Hemosil SynthA-Fox-SF. Twenty of 109 patients had long level of APTT by measuring Hemosil synhAsil-SS reagent and Hemosil SynthA-Fox-SF. Summary/Conclusions: Haemostasis is a complex physiological cascade which was began at the endothelium injury. Many kinds of complex procedures occurred with purpose to control bleeding. Because of this complex cascade pathway, bleeding is not occurred every kind of APTT level. Bleeding events usually occurred at <30 level of plasma factors but mild or moderate plasma factor levels can caused bleeding. Therefore sensitive of APTT reagents are very important. Every kind of APTT reagent do not have equal sensitivity against plasma factor levels, phospholipid composition and concentration in thromboplastin reagent. Several studies suggested that range of APTT should be determine according to the devices and reagents and also several studies comparied APTT reagents which was included silica, ellagic acid and phospholipid by composed of synthetic or animal organinated and several studies determined a target level of APTT for looking at the plasma factors levels. If we want to get a correct result of APTT, ranges of APTT must be determine according to reagents which was used in APTT devices and APTT reagents must be sensitive against borderline cases who had a mild or moderate low levels of factors and the presence lupus anticoagulant. We need further studies to make a standardization and harmonization of APTT.
FOBT was positive in only 65 (30.23%) subjects. 170 (79.06%) patients under-
Apo B, white cell count, and platelet count all impose risk of thromboembolism, further work exploring the associations and impacts of these parameters on the development of cardiovascular diseases should be mandatory.

**PB2094**

UNUSUAL DISTRIBUTION OF INTERLEUKIN-10 C-592A GENE POLYMORPHISM IN PATIENTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA FROM NORTH-WESTERN RUSSIA

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**Background:** Primary immune thrombocytopenia (ITP) is a rare hematological disease with unknown etiology. It is characterized by heterogeneity of the laboratory parameters as well as the features of clinical manifestation. DNA polymorphism of several cytokine genes has been suggested to modulate the risk of ITP development or/and treatment response in distinct population groups. There is no data on the prevalence of cytokine gene polymorphisms in ITP patients from the North-Western region of Russia (NWR).

**Methods:** To establish the features of genotypes distribution for several cytokine promoter gene polymorphisms in ITP patients from NWR.

**Results:** A total of 68 patients (59 women and 9 men) with chronic primary ITP were involved in the study. The median age of the group was 57 years (range: 24–77). The mean duration of ITP was 7 years (2–48). In 19 (32.2%) women, ITP was diagnosed before 30 years old; 26 (38.2%) patients (5 men and 21 women) were diagnosed at age 30–50 years; 23 (33.8%) patients (9 men and 19 women) developed ITP after 50 years old. The control group consisted of 240 healthy persons originated from NWR. Nucleotide variations in the genes coding for interleukin (IL)-1β (-31T/C), IL-6 (-174G/C), IL-10 (-592C/A) and tumor-necrosis factor alpha (TNFA -308 G/A) were discriminated by PCR and subsequent restriction analysis (PCR-RFLP). Intergroup differences in genotype frequencies were assessed by Fisher’s exact method. Odds ratios (OR), their 95% confidence intervals (CI) and p-values were calculated by using the GraphPad Prism 5.0 software.

**Results:** The frequency of the IL-10 -592CC genotype was slightly increased in the ITP group when compared to controls (46.0% in control group; OR=3.5, 95% CI: 1.1-14.2, p=0.044). On the contrary, in the group of affected men we observed the increase of persons who had IL-10 -592A allele (75.0% vs 46.0% in control group; OR=3.5, 95% CI: 0.7-18.3, p=0.15). Genotype frequencies for other studied genes were similar between the patients and control group as well as between women and men with ITP. We have also found almost 2-fold increase of the IL-1b -31CC frequency in women diagnosed before 30 years old compared to other patients (15.8% vs 8.2% respectively; OR=2.1, 95% CI: 0.4-10.5, p=0.39). The presence of the TNFA -308A allele was more often seen in patients diagnosed before 50 years old (26.7% vs 8.7% in other ITP patients; OR=3.8, 95% CI: 0.8-18.8, p=0.12).

**Summary/Conclusions:** We suggest that the IL-10 -592CC genotype is associated with increased risk of ITP from women in NWR. On the other hand, the IL-10 –592A allele could be involved in pathogenesis of ITP in men. Further studies are needed to clarify the significance of TNFA and IL-1b gene polymorphism in ITP development.

**Platelets disorders**

**PB2095**

COMBINED TREATMENT OF AZATHIOPRINE AND ROMIPLOSTIM IN PATIENTS ITP REFRACTORY TO STEROIDS OR THROMBOPOIETIN ANALOGS

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**Background:** More than 70% of patients with Immune Primary Thrombocytopenia (ITP) respond to steroids, but 40 to 70% relapse in the first year follow-up. The use of romiplostim in this group is effective, although 5% failure has been described. In recent literature, there are clinical case reports series describing the potential effect of combined treatment with thrombopoietin analogues and immunosuppressive drugs such as steroids, cyclophosphamide and rituximab. We have not found references to the combined use of azathioprine (AZA) and romiplostim (ROM).

**Aims:** To describe our experience in the combined use of azathioprine and romiplostim as a rescue treatment in patients with acute or newly diagnosed ITP refractory to corticosteroids or corticosteroid-dependence and refractory to maximal doses of romiplostim monotherapy.

**Methods:** We analyzed patients with newly diagnosed or persistent ITP, with corticosteroid-dependence or refractory to steroids and refractory to romiplostim, both in monotherapy. We have considered refractoriness to steroids not reaching platelets higher than 30x109/L. Corticosteroid-dependence as the need for ongoing or repeated doses administration of corticosteroids for at least 2 months to maintain a platelet count at or above 30 x109/L and/or to avoid corticosteroids withdrawal. We considered refractoriness to romiplostim not get platelets greater than 30x109/L with 10mg/kg/week for at least 3 consecutive weeks. All patients have been diagnosed in a single center with the same physician responsible for the treatment and follow-up. The initial doses of AZA was 100mg/days (2mg/kg/day) and ROM 10mcg/kg/week. Patients have been evaluated every week until platelets were higher than 30x109/L for consequtive weeks, after this they were reviewed monthly.

**Results:** We treated 4 patients (75% female) with a median age at diagnosis of ITP of 53 years old (RIQ, 20-61 years). Treatments received prior to the use of the combination of AZA and ROM were polyclonal immunoglobulins (Ig), corticosteroids, methotrexate or placebo and romiplostim. Responses to steroids and romiplostim in monotherapy were: Mediane dexamethasone cycles (40mg/days x 4 days) was 2.5 (2-4 cycles, IQR). The initial dose of prednisone was 1-2mg/kg/days with a median treatment day of 31.5 days (28-60 days, IQR). The type of response to steroids was PR with corticosteroid-pendence in one patient, 3 patients NR. Median time from ITP diagnosis and romiplostim indication was 9.5 weeks (7-48 weeks, IQR). Median platelet count at the start of romiplostim was 6x109/L (2-13x109/L, IQR). The median platelet count achieved at maximal doses of romiplostim for at least 2 consecutive weeks was 10x109/L (3-19x109/L, IQR). Once established the refractoriness to romiplostim, we maintained ROM 10mcg/kg/week and AZA was initiated at 100mg/day. The median time from romiplostim indication to the association with azathioprine was 9.8 weeks (5.5 to 15 weeks, IQR). The median time to response after initiation of combination of AZA and ROM was 21 days (15-35 days, IQR). The types of response were: • One patient did not respond after 60 days of therapy. 
• 1 patient with refractory to corticosteroids at 7 months in the absence of active treatment. The combined was necessary during 6 months.
• 2 CRs still undergoing combined dose reduction (current dose romiplostim 2mcg/kg/week and azathioprine 50mg id). Median platelets from onset of dose reduction 169x109/L (128-176x109/L, IQR). Duration of RC, 7 and 14 months. Non adverse events have been described in combination treatment.

**Summary/Conclusions:** The use of azathioprine and romiplostim in combination could be a safe and effective alternative in subjects refractory to steroids or corticosteroid-dependence and thrombopoietin analogs alone. More studies are needed to clarify the mechanism of complementation between the two drugs.

**PB2096**

AGONIST-INDUCED PLATELET REACTIVITY CORRELATES WITH BLEEDING IN HEMATO-ONCOLOGICAL PATIENTS

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**Background:** Prophylactic platelet transfusions are administered to prevent bleeding in hematological patients. However, bleeding still occurs, despite these transfusions. This practice is costly and not without risk. Better predictors of bleeding are needed and flow cytometric evaluation of platelet function might aid the clinician in identifying patients at risk of bleeding. This evaluation can be performed within the hour and is not hampered by low platelet count.
Aims: Our objective was to assess a possible correlation between bleeding and platelet function in thrombocytopenic hematopoietic patients.

Methods: Inclusion was possible for admitted hematopoietic-oncology patients aged 18 years and above after written informed consent. Furthermore, an expected need for platelet transfusions was necessary. Bleeding was graded according to the WHO bleeding scale. Platelet reactivity to stimulation by either adenosine diphosphate (ADP), crosslinked-collagen-related peptide (CRP-XL), PAR-1 or PAR4-activating peptide (AP) was measured using flow cytometry.

Results: A total of 114 evaluations were available from 21 consecutive patients. Platelet reactivity in response to stimulation by all four studied agonists was inversely correlated with significant bleeding. Odds Ratio’s (OR) for bleeding were 0.28 for every unit increase in median fluorescence intensity (MFI) [95% Confidence interval (CI) 0.11-0.73] for ADP; 0.59 [0.40-0.87] for CRP-XL; 0.59 [0.37-0.94] for PAR1-AP and 0.43 [0.23-0.79] for PAR4-AP. The platelet count was not correlated with bleeding (OR 0.99 [0.96-1.02]).

Summary/Conclusions: Our research indicates that platelet reactivity is significantly correlated to bleeding. Platelet function testing could provide a basis for a personalized transfusion regime, in which platelet transfusions are limited to those at risk of bleeding.

PB2097 TUMOR NECROSIS FACTOR-A AND TUMOR NECROSIS FACTOR-B SINGLE NUCLEOTIDE POLYMORPHISM AND CHRONICITY IN EGYPTIAN PEDIATRIC PATIENTS WITH IMMUNE THROMBOCYTOPENIA N. El-Gharawi1, G. Shahn1, A. Eid1, N. Dia1, M. El-Ghamrawy2,3
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Background: Although the etiology of immune thrombocytopenic purpura (ITP) remains unclear, both genetic and environmental factors may contribute to the development of disease. Tumor necrosis factor alpha & beta (TNF-α and TNF-β) are pro-inflammatory cytokines that play a role in regulation of cell differentiation, proliferation and death, as well as in inflammation, innate and adaptive immune responses, and have been implicated in a wide variety of human diseases. We hypothesized that inflammatory cytokine genes polymorphisms (TNF-α and TNF-β) in ITP pediatric patients may play a fundamental role in pathogenesis of the disease. We aimed to evaluate whether polymorphisms of the TNF-α and TNF-β genes might be the base for future specific immunomodulatory therapies for chronic ITP (cITP) in children.

Aims: The current case-control study aimed at detecting TNF-α (-308 G/A) and TNF-β (+252 A/G) genes polymorphism in Egyptian children with cITP and studying their possible association with chronic evolution of the disease.

Methods: The current study included 80 Egyptian cITP patients at Pediatric Hematology Unit, Cairo University (mean age 7.08±3.64 years) and 100 matched unrelated healthy controls. Genotyping was performed using polymerase chain reaction restriction fragment length polymorphism technique (PCR-RFLP).

Results: TNF-α genotyping revealed that wild G/G, heterozygous G/A and homozygous A/A genotypes among cITP patients were 81.2%, 15% and 3.8% respectively versus 79%, 20% and 1% in control group, while TNF-β wild A/A, heterozygous A/G and homozygous G/G genotypes among cITP patients were 55%, 40% and 5% respectively versus 60%, 28% and 12% in control group, with no statistically significant difference between both groups. Patients having homozygous TNF-α genotype showed statistically significant higher mean age, longer disease duration & lower mean platelet count (p=0.005, 0.024 and 0.008 respectively). TNF-α polymorphism was more frequent among unresponsive patients compared to responsive patients with statistically significant difference. Calculated risk estimation revealed that combined genes polymorphism conferred three fold increased risk of development of cITP (OR=3.491, 95% CI: 1.235-9.869, p=0.015).

Summary/Conclusions: We hereby report a strong association between combined polymorphisms of both TNF-α & TNF-β genes and susceptibility to chronicity of ITP in Egyptian children. Further studies for gene polymorphisms which could affect the pathogenesis of ITP and facilitate the development of new therapeutic modalities are recommended.

PB2098 PROGNOSTIC FACTORS IN PRIMARY IMMUNE THROMBOCYTOPENIA OF CHILDHOOD A. Gkoutsi1, T. Palianopoulos1, E. Pappas2, E. Papapetrou3, C. Tsouisi3, N. Chaliassos5, A. Makis1
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Background: Primary immune thrombocytopenia (ITP) is an immune disorder with varied course. According the duration of the disease, it is distinguished in newly diagnosed (<3 months), persistent (3-12 months) and chronic (>12 months). International studies have highlighted prognostic factors for each form of ITP. Very similar studies have yet to be conducted in Greece.

Aims: The evaluation of clinical and laboratory parameters and the identification of prognostic markers for the three forms of the disease in children with ITP from an academic reference center in Greece.

Methods: This retrospective study included 57 children with ITP in the past 13 years, aged 1-16 years (median age 5.2). The following data were recorded: age, gender, preceding infection, bleeding type, duration of symptoms and platelet count at the diagnosis, treatment, disease course and immunological markers and comparison was made among the three types of ITP.

Results: 39 children had newly diagnosed, 4 had persistent and 14 had chronic disease. Due to the small number of children with persistent form they were incorporated in the group of children with newly diagnosed ITP. In chronic ITP children are more likely be above 10 years of age (p=0.015) and to have gradual initiation of the disease (p=0.001) compared with newly diagnosed/persistent group (57% vs 21% and 79% vs 9%, respectively). Recent history of infection was found mainly in newly diagnosed/persistent group (70% vs 21%, p=0.013). Platelet count below 10 x 10^9/L at diagnosis was found more frequently in newly diagnosed/persistent group (79% vs 36%, p=0.01). Similar, but not statistically significant difference, was found with mucosal bleedings (70% vs 50%, p=0.81). Children with newly diagnosed/persistent disease had less frequently impaired immunological markers (12% vs 65%, p=0.013). Children with newly diagnosed/persistent disease were more likely to be receiving intravenous gamma globulin and/or corticosteroids (p=0.05). None of the children exhibited severe spontaneous bleeding.

Summary/Conclusions: Even though ITP in children is usually a self-limited disease, with rare serious bleeding complications, the newly diagnosed/persistent and the chronic form of the disease are characterized by different predictive parameters that can be used in clinical practice.

PB2099 CANCER-ASSOCIATED IMMUNE THROMBOCYTOPENIA G. Pinto1, *, P. Herrera1
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Background: Cases of cancer-associated immune thrombocytopenia (IT) have been reported recently, but there are few reports and case series that describe clinical features and response to treatment.

Aims: We report our experience of 10 years at a single hospital in Spain, in patients with IT concurrent with neoplasia.

Methods: We identified the patients by data search of hospital records from 2006 to 2016, with diagnosis of IT with previous diagnosis of cancer, not related with chemotherapy or radiotherapy, not suggestive of bone marrow infiltration, drug-induced, infection of disseminated intravascular coagulation. For the diagnostic examination of number of children with IT was not mandatory.

Results: The two most common cancers associated with IT were bladder and lung neoplasms, but the occurrence of prior cancer (third part of patients) was not uncommon. The IT can appear at any stages of cancer, and it is mainly detected at the first two years after the diagnosis when the patient have been in acceptable antitumoral response. They usually manifest with very low platelet count <20,000, but not always with evident clinical bleeding. The response to therapy was fast and complete with corticoids (usually in the first week) in the majority of patients, but some cases require the combination second line with immunoglobulins or thrombopoietin receptor agonists, and in the follow-up, the response was persistent without recurrence in the first year post-treatment (Table 1).

Table 1.

PB2100 THE ROLE OF MEAN PLATELET VOLUME IN NEONATAL SEPSIS: AN RETROSPECTIVE CASE CONTROL STUDY IN A LEVEL III NEONATAL INTENSIVE CARE UNIT A. Srinivasan1, *, A. Meer1, M. Marron-Corvin1
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Summary/Conclusions: The CAIT is a rare hematological paraneoplastic syndrome that occur in solid tumors, usually associated to low platelet count but not always with life-threatening bleeding, requiring therapy with corticosteroids as first line, and generally related with a benign clinical course with a rapid and persistent response.
Background: Sepsis is a relatively common diagnosis in the neonatal period. Apart from blood cultures which are the gold standard, C-reactive protein (CRP), total white blood cell count (WBC) and the ratio of immature to mature neutrophils (I:T) are considered to be useful markers of sepsis in the neonatal period. There are a few studies that show that mean platelet volume (MPV) is elevated in infectious disease processes.

Aims: The aim of this study was to investigate whether mean platelet volume is increased in neonates with sepsis.

Methods: Only term neonates were included in the study. Exclusion criteria included: (a) Any neonate born with a genetic defect, (b) Any neonate with suspected immunodeficiency, (c) Any neonate requiring surgery in the post-natal period, (d) Neonates admitted to NICU for hyperbilirubinemia, (e) Neonates requiring extensive resuscitation at birth resulting in documented Hypoxemic Encephalopathy or requiring transfer to a Regional Perinatal Center. Medical records were reviewed from March 2015 to June 2016 and a total of 114 eligible neonates were included in the study and they were divided into 2 groups: Sepsis (defined as clinical sepsis, defined by either culture positivity and/or clinical features plus treatment with antibiotics exceeding 48 hours) and 75 healthy controls (as defined by neonates in whom antibiotics were never started or discontinued when cultures were negative for 48 hours and the absence of clinical features of sepsis). Total white blood cell count, C-reactive protein, immature to total neutrophil count and mean platelet volume (defined as the product of platelet count and mean platelet diameter) were compared on two occasions (first within 24 hours and the second between 24 to 48 hours after delivery) were compared between the two groups.

Results: There was no statistically significant difference in the mean platelet volume between the study group and the control group (p value 0.9 in the first 24 hours and p value 0.6 in the 24-48 hours sample). There was, however, a statistically significant difference between immature to total neutrophil count and C-reactive protein on both samples (p value <0.0001) (Table 1).

Summary/Conclusions: In our study there was no statistically significant difference in the mean platelet volume values between neonates with sepsis and healthy controls. C-reactive protein and immature to total neutrophil count continue to be reliable markers of neonatal sepsis.

PB2101
IS PLATELET TRANSFUSION WARRANTED IN PATIENTS WITH ACUTE TTP REQUIRING CENTRAL VENOUS CATHETER INSERTION? R. Low1,*, T. Dutt2
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Background: Thrombotic thrombocytopenic purpura (TTP) has a high mortality rate. The cornerstone of management is plasma exchange (PE) which usually requires urgent insertion of a central venous catheter. Patients often have a platelet count of <50x109/L at presentation however, National BCSH Guidance advises against platelet transfusion in TTP due to the perceived high aggregability state and risk of associated fatal thrombosis. The risk of thrombocytopenia related haemorrhage however causes anxiety and dilemma for the team responsible for line insertion and may lead to delays or unnecessary platelet transfusion.

Aims: The aim of the study is determine the average platelet count at time of line insertion for patients requiring central line insertion and to assess bleeding complications are observed.

Methods: We retrospectively reviewed all central venous catheter lines inserted in patients presenting to a regional TTP Centre over a 4-year period from 2012-2016.

Results: A total of 48 patients confirmed to have TTP with an ADAMTS13 <5% underwent line insertion: 94 central venous catheter lines were inserted: 40% femoral, 60% internal jugular vein. The median number of lines inserted per patient episode was 3, with a range of 1-5. Median presenting platelet count for first line insertion was 25x109/L (IQR 9-26 x109/L). 70% of lines were inserted by critical care and the remaining 30% by interventional radiology. Platelet transfusions were not administered pre line insertion and all line insertion related bleeding complications were documented during or after line insertion. 5 patients had ‘excessive oozing at the insertion site’ documented, within the first 24 hours of insertion, for which no intervention was required. There were no deaths related to line insertion.

Summary/Conclusions: In conclusion, this study shows no significant bleeding risk associated with central venous catheter insertion in thromboticocyanic patients presenting with TTP. The results support guidance against prophylactic platelet transfusion in this setting and provide reassurance for teams tasked with central line insertion in this critically unwell patient group.

PB2102
LONG-TERM EFFICACY AND SAFETY OF THROMBOPOIETIN AGONISTS IN ADULT REFRACTORY CHRONIC IMMUNE THROMBOCYTOPENIA M. Kaliou1,*, E. Gavriilaki1,*, G. Papaioannou1, Z. Bousiou1, M. Iskas1, C. Vadikoliou1, C. Lalayanni1, A. Athanasadou1, R. Saloum1, A. Anagnostopoulou1
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Background: Management of chronic immune thrombocytopenia (cITP) aims not only to increase and maintain platelet counts in safe levels, but also to improve quality of life. Thrombopoietin agonists eltrombopag and romiplostim have been approved in refractory ITP. The lack of randomized studies allows only for real-world data comparison on the two agents.

Aims: In the present study we evaluate and compare long-term efficacy and safety of eltrombopag and romiplostim in clinical practice and assess the switching feasibility between the two agonists.

Methods: Treatment with thrombopoietin agonists was initiated in 20 adult patients (pts) with refractory cITP between June 2011-2016. Patients resistant or intolerant to the first agonist switched to the second one. Complete response (CR) was defined as a platelet count of ≥100x109/L.

Results: Ertrombopag was administered in 15 pts, 6 male:9 female with a median age of 46 years (19-76 yrs) for 13 months (1.4-54 mo). Patients had received a median of 1 previous treatment (range 1-7); corticosteroids (15/15), intravenous immunoglobulin (5/15), rituximab (2/15), vincristine (1/15), cyclophosphamide (2/15), romiplostim (2/15), danazol (1/15) and splenectomy (1/15). Before eltrombopag treatment, the majority (8/15) showed grade 4 (WHO) thrombocytopenia. Initial dose was 50 mg and increased to 75 mg daily in 3/15 pts and in combination with corticosteroids that were gradually tapered by the 5th week in 12/15. Median platelets value by the 2nd week of administration was 140x109/L (5-450 x109/L); whereas, by the 4th week increased to 185x109/L (16-500x109/L). At the end of follow-up, all patients but one achieved CR with median platelets of 145x109/L (60-400 x109/L). Regarding adverse events, 1/15 pt presented hemolytic anemia, 1/15 pt hepatotoxicity grade 2 with episodes of thrombocytopenia grade 4 and 1/15 pt pulmonary embolism during the second month of treatment. The latter 2 pts switched to romiplostim. Romiplostim was administered in 9 pts, 4 male:5 female with a median age of 46 years (33-67 yrs) for 6.7 months (4-60 l). They had received a median of 3 previous treatments (range 1-8); corticosteroids (9/9), intravenous immunoglobulin (6/9), rituximab (6/9), vincristine (2/9), cyclosporine (2/9), eltrombopag (2/9), danazol (1/9) and splenectomy (2/9). The majority (5/9) presented thrombocytopenia grade 4 before romiplostim. Median platelets number by the 2nd week of administration was 50x109/L (9-140 x109/L); whereas, by the 4th week increased to 115x109/L (20-400x109/L). At the end of follow-up, 6/9 pts achieved CR with median platelets at 145x109/L (110-400x109/L). All patients received concomitant steroid treatment that was gradually tapered and stopped in 6/9 pts. 2/9 pts switched to eltrombopag due to thrombocytopenia grade 4 and 1/9 pt to danazol and low-dose steroids achieving CR. No adverse events associated with romiplostim treatment were reported. No significant differences were found between the 2 treatment groups. All 4 patients that switched to the other agonist achieved CR without adverse events.

Summary/Conclusions: Our real-world data suggest that both eltrombopag and romiplostim are safe, well tolerated and highly effective in refractory cITP and furthermore, switching to another agonist is safe and effective. Future studies will determine predisposing factors for adverse events and more accurate classification of patients that will allow for better treatment guidance.
D receptor (VDR) polymorphisms in the development of autoimmune diseases. Vitamin D affects both innate and adaptive immune responses that have been blamed in immune thrombocytopenia (ITP) pathogenesis.

Aims: The aim of this study is to associate the vitamin D receptor gene polymorphism BsmI in cases of adult primary immune thrombocytopenia. Methods: Vitamin D receptor polymorphism BsmI (rs1544410) was detected by Polymerase Chain Reaction followed by Restriction Fragment Length Polymorphism (PCR–RFLP). Deoxynucleic acid (DNA) samples were extracted from peripheral blood of 40 ITP patients and 60 genetically and ethically matched healthy controls.

Results: Statistically significant difference was found in the BsmI polymorphism between ITP patients and controls (χ² = 8.77, P value = 0.01). The BsmI polymorphism B allele was higher in ITP group than that in controls but in statistically insignificant difference (χ² = 2.125, P = 0.145). Bb genotype played a protective role in ITP incidence.

Summary/Conclusions: This is the first published report on VDR gene polymorphisms in adult primary ITP patients. The BsmI genotype was associated with increased risk for ITP incidence with no obvious effect on bleeding severity, platelet count nor site of bleeding.

PB2104

A SURVEY OF THE TREATMENT OF THE PREVENTION OF NAIT IN THE UK AND IRELAND

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Background: Neonatal alloimmune thrombocytopenia, (NAIT) is caused by maternal antibodies generated against alloantigens carried on fetal platelets, which cross the placenta and induce destruction of platelets in the fetus. In most cases the maternal immunisation is triggered by exposure to fetal platelets at delivery. As a result, the clinical presentation tends to be more severe in subsequent pregnancies. Recent studies and guidelines have suggested that intravenous immunoglobulin (IVIG) with or without steroids can significantly reduce the severity of thrombocytopenia in subsequent pregnancies.

Aims: We set out to establish if there is consistency in the management of the prevention of NAIT across Ireland and the United Kingdom (UK).

Methods: A survey was set up on Survey Monkey and all members of the UK-Ireland Haematology group were contacted by email with a link to the survey in January 2015. In total 90 individual Specialists were contacted across 70 centres.

Results: 30 responses were received to the following questions. Who manages the prevention of NAIT in your centre? 34% of respondents stated that it was managed jointly by haematologist/feto-maternal specialists, with 26% responding it was overseen solely by haematologists and 40% solely by feto-maternal specialists. Secondly what risk stratification each respondent used to decide risk of NAIT in the current pregnancy? 82% stated that they took into account multiple risk factors but 18% stratified risk based only on the outcome of previous pregnancies. Thirdly how many groups do you define after risk stratification? 60% identified 3 strata of risk (standard, high and very high) with 40% classifying two risk groups (standard versus high risk). Fourthly respondents outlined their management of a standard risk group defined as confirmed thrombocytopenia with antibody. 43% give IVIG 1g/kg weekly from 20 weeks, 28% give 1g/kg from 20 weeks to 32 weeks with 1g/kg at 32 weeks starting 1g/kg with IVIG from 20 weeks. 23% referred to feto-maternal specialist to decide IVIG. Just 20% give 0.5mg/kg of steroids from 20 or 32 weeks. For high risk pregnancies defined as confirmed antibody positive with previous intracranial haemorrhage (ICH) after 28 weeks: 36% of centres give IVIG 1g/kg from 20 weeks, 36% give 1g/kg from 20 weeks increasing to 2g/kg at 32 weeks with 14% giving 2g/kg from 20 weeks and 14% initiating at 12 weeks. 40% gave 0.5mg/kg of steroids from 12-32 weeks starting. 60% of centres use a very high risk protocol (ICH before 28 weeks) with more intensive IVIG starting at 12 or 20 weeks with steroids of variable intensity and duration. Finally respondents were questioned whether there was a planned delivery time and method for the pregnancy? 58% plan a delivery at 38 weeks with no specific delivery mode. 18% plan delivery at 38 weeks by caesarean section, 8% plan a caesarean section but with no set time and 16% have no specific protocol plan for delivery.

Summary/Conclusions: The results of this survey reveal that the optimal medical management for the prevention of NAIT, namely the medication, dosage and schedule vary widely reflecting the lack of good evidence to guide centres in this very challenging area. Based on this survey we plan with our colleagues to set up a standard and evidence-based protocol for the management of NAIT across Ireland and the United Kingdom (UK).

PB2105

THE EVALUATION OF REACTIVE OXYGEN SPECIES IN CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA AND HELICOBACTER PYLORI INFECTION VERSUS CHRONIC ITP WITHOUT HELICOBACTER PYLORI INFECTION

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Background: Chronic idiopathic thrombocytopenic purpura (ITP) is an acquired disease characterized by a low platelet count caused by an immunological peripheral platelet destruction or a decreased platelet production. Several studies have shown increased reactive oxygen species (ROS) levels in chronic ITP and also a possible association between Helicobacter pylori (H. pylori) infection and immunological peripheral platelet destruction. Aims: To evaluate whether patients with chronic ITP and H. pylori infection exhibit higher ROS levels compared to patients with chronic ITP and no H. pylori infection and whether there are statistically significant differences between the two groups.

Methods: We studied 29 patients with chronic ITP (median age 39 years) hospitalized in the Clinic of Hematology, Filantropia City Hospital, Craiova, Romania, between 2014 and 2016 (informed consent obtained). All patients were diagnosed with ITP, other causes of thrombocytopenia having been ruled out by bone marrow aspiration. The patients were divided in two groups: patients with ITP and H. pylori infection (group A) and patients with chronic ITP without H. pylori infection (group B). Two groups were used to identify the presence of a H. pylori infection and reactive oxygen species were evaluated by FORT (Free Oxygen Radicals testing) test from a single drop of capillary blood, at the time of diagnosis, before the administration of any drug (the normal value of FORT is considered less than 2.3 mmol/l H2O2), using a CR3000 analyzer (Callegari SpA, Parma, Italy). The differences between the two groups were assessed using the Student T-test and a p-value of less than 0.05 was considered statistically significant.

Results: Group A consisted of 11 patients positive for H. pylori, whereas group B included 18 patients with no H. pylori infection. ROS levels, measured by the FORT test, were elevated in both groups (2.8 – 3.6 mmol/l H2O2). However, statistically significant differences were found in favour of group A, with higher ROS values than group B. The A group also associated lower platelet counts and more patients pertaining to this group relapsed in comparison to group B.

Summary/Conclusions: In chronic ITP, increased levels of ROS are associated with elevated aut oantibody production. Autoantibodies are involved in platelet destruction via highly a immunogenic activity. On the other hand, association of H. pylori infection, via chronic inflammation, led to a supplementary increase in ROS levels and increased platelet destruction.

PB2106

IMMUNE THROMBOCYTOPENIA AND PREGNANCY: A SPANISH CASE SERIES OF 270 PREGNANCIES IN PRIMARY ITP


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Background: Effect of pregnancy on the course of primary immune thrombocytopenia (ITP) is not well known. Besides, due to the lack of clinical assays, it is difficult to predict outcome of treatment. There are no studies on the outcomes of ITP during pregnancy. Also, data about outcome predictors of pregnancies and neonates born to mothers with ITP is scarce.

Aims: To evaluate management and results of pregnancy and delivery on pregnant ITP women and on their offspring.

Methods: All women diagnosed of primary ITP (according to international consensus criteria) from 2011 to 2016 in 23 Spanish Hematology Departments who had at least one pregnancy after ITP onset were included in this registry.

Results: We included 270 primary ITP pregnancies from 184 women. At pregnancy diagnosis, we observed a majority of chronic ITP cases (71.4%). At ITP diagnosis, we observed a majority of chronic ITP cases (71.4%). At ITP diagnosis as a median. ITP diagnosis as a median. ITP diagnosis as a median.

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50.8% of women received corticosteroids, immunoglobulins (IVIG) (16.9%), rituximab (8.8%) and splenectomy (8.4%) as ITP treatments between or before new pregnancies. On the other hand, 26.4% of women needed treatment for ITP during pregnancy, mainly steroids (13.5%) and IVIG (10.2%). The median platelet-count nadir during pregnancy was 74 x 10^9/l (IQR: 36-172). 127 (47%) pregnancies suffered from non-haemostatic platelet levels (less than 50 x 10^9/l) with 73 (27.0%) women who achieved less than 30 x 10^9/l. 56 (20.7%) women exhibited hemorrhagic symptoms, being 30 (11.1%) of them severe bleedings.

Regarding type of delivery, this was vaginal in 63.4% of pregnancies and cesarean sections 36.6%. Median platelet count at delivery was 110 x 10^9/l (IQR: 79-181). 43 patients (23.4%) experienced 57 bleeding episodes. We only observed 48 cases (20.4%) of neonatal thrombocytopenia among 235 living newborns.

Summary/Conclusions: Our results are comparable to previously reported studies. No severe bleeding complications during pregnancy and/or delivery were observed in our case series. Rate of neonatal thrombocytopenia, and therefore, newborn bleeding is low.

PB2107

ANALYSIS OF THE DEMOGRAPHIC, CLINICAL, LABORATORY AND TREATMENT-RELATED DATA OF ITP PATIENTS IN GREECE BASED ON THE NATIONAL ITP REGISTRY OF THE HELLENIC SOCIETY OF HAEMATOLOGY


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Background: Immune thrombocytopenia (ITP) consists of various acquired disorders caused by autoantibodies against platelets resulting in increased platelet destruction and impaired thrombopoiesis. ITP is characterized as primary when an underling etiology cannot be identified and secondary when a certain etiology exists. Data concerning ITP characteristics at a national level are limited.

Aims: The purpose of the study was to access systematically the demographic, clinical, laboratory and treatment-related data of ITP in Greece based on the national database (ITP registry) operated and supported by the Hellenic Society of Haematology.

Methods: A total of 2215 patients data were collected over 2013-2016. The data source is a unique database initiated and managed by the Haematology Department of the University of Crete (UoC) and supported by the Center of Information and Communications Technologies of the UoC. The registry has been configured and a wide variety of symptoms are seen on initial presentation.

Aims: A retrospective review of the significance of specific symptoms and their duration on mortality.

Methods: A retrospective review of all consecutive admissions to a single tertiary care center between 2009 and 2015. Only patients who required plasma exchange were included. Patients’ symptoms and their duration were reviewed in addition to presenting anti-ADAMTS13 IgG antibody levels and ADAMTS13 antigen levels, both of which have previously been found to have prognostic significance.

Results: 106 patients (68% female) were included with a median age of 48.58 years. 58% were Caucasian and 19.8% Afro-Caribbean. The mortality rate was 7.4% (n=8). 47% of patients had neurological symptoms on presentation, 24% reported a bleeding history and 12% a recent infection. The most common presenting symptoms were headache (27.4%), bleeding (24%) spontaneous bruising/pcutaneous rashes (19.8%). The anti-ADAMTS13 IgG level was not however significantly higher in these symptoms when compared to others (Table 1) suggesting microangiopathic thrombosis location plays an important role in TTP prognosis. The median duration of symptoms prior to presentation was 7 days (range 1-80 days). 8.5% of patients were transferred as emergency admissions. One third of TTP patients in the highest quartile for symptom duration (>10 days) had significantly higher anti-ADAMTS13 IgG antibody levels than those in the lowest quartile for symptom duration (<2 days) (65% vs 26%, p=0.002) and may have increased mortality (symptoms < 2 days mortality 7.4%, symptoms > 10 days 14.3%, p=0.19).

Summary/Conclusions: Whilst there is little difference in the anti-ADAMTS13 IgG antibody and ADAMTS13 levels seen with symptom differences, there is a wide disparity in terms of mortality suggesting the effect of microangiopathic thrombosis differs by location. Abdominal pain, not previously recognized as a serious presenting symptom in TTP, is an ominous diagnostic feature although this should be interpreted with caution given the sample size. Anti-ADAMTS13 IgG antibody level increases with symptom duration and this may lead to increased mortality.
PB2109
NOVEL TECHNIQUES FOR MONITORING GALNZMANN THROMBASTHENIA PATIENT UNDERGOING SURGICAL INTERVENTIONS
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Background: Glanzmann thrombasthenia (GT) patients undergoing surgical procedures are often treated by platelet transfusion. However many GT patients who have been previously exposed to platelets may form antibodies either against the missing αIIbβ3 antigen or directed against MHC-class molecules thus hampering the efficacy of the procedure. Because of the rarity of disease there is paucity of data regarding platelet transfusion protocols during the perioperative period. We herein describe our experience with monitoring the proportion of donor platelets following transfusion, and their contribution to whole blood clot formation.

Aims: To describe the use of flow cytometry (FC) analysis in order to detect donor transfused platelets in a GT patient undergoing a minor surgical procedure and to assess the correlation between FC analysis and the results of Rotational thromboelastography (ROTEM).

Methods: A nine year old female patient with GT underwent teeth extraction. The patient received platelet transfusion around the procedure. Complete blood counts, ROTEM, FC to detect the number of donor platelets and their ADP dependent activity, were sampled and followed till 7 days post teeth extraction.

Results: Prior to teeth extraction upon injection of local anesthetics patient developed a buccal hematomata probably owing to local blood vessel penetration. The patient did not experience any post extraction bleeding. Hematoma was absorbed within several days. Post transfusion platelets FC demonstrated 20.6% donor platelets equivalent to 55,620 donor platelets. Platelets activation was determined following ADP addition by examination CD62 antigen expression. Seven days post platelet transfusion FC demonstrated 2.6% equivalent to 8,658 donor platelets. The decline in the number of active platelets was associated with a reduced clot firmness (MCF) and lower α-angle as assessed by ROTEM (Figure 1).

Figure 1.

Summary/Conclusions: Clinical decision making in patients with GT may be aided by application of novel techniques, evaluating the number of active donor platelets and actual clot formation. This data may help making more knowledgeable decisions as for the need for further platelet transfusion or for the need for rFVIIa. Thus leading to improved monitoring and better patients’ care.

PB2110
CAN HISTOCHEMICAL C-MPL POSITIVITY IN BONE MARROW BE A PREDICTOR FOR SPLENECTOMY IN IMMUNE THROMBOCYTOPENIA?
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Background: Splenectomy is used as the second line therapy in patients with immune thrombocytopenia (ITP). However, there is no parameter predicting splenectomy indication.

Aims: Aim of the present study was to evaluate immune histochemical Cloned Myeloid Leukemia Virus (c-mpl) positivity in bone marrow specimens of ITP patients with or without splenectomy indications.

Methods: Bone marrow specimens were taken from 24 patients who were diagnosed with ITP and who had splenectomy (15 female, 9 male, mean age 50±16) before splenectomy and 30 patients who were diagnosed with ITP but did not have splenectomy (15 female, 15 male, mean age 52±19). c-mpl staining was carried out retrospectively. Immunohistochemical (IHC) staining using Avidin-Biotin complex system (ABC) was conducted. For IHC, sections prepared from blocks were taken onto poly-L-lysine coated slides (MicroSlides Snowcoat X-tra, Surgipath, Richmond, IL, USA) and kept in an incubator at 37 °C overnight. Dissections were treated with ABC complex (Santa Cruz/excitrite 13181-500) stain. Cytoplasmic and nuclear staining was observed in megakaryocytes using IHC c-MPL and vitamin D. Evaluation was made based on the intensity of the staining; i.e. negative (0), weak (1+), moderate (2+) and strong (3+) (1). All patients who had splenectomy were in chronic phase of the disease. The present study was supported as a Scientific Research Project by Adnan Menderes University (TPF-15027).

Results: c-mpl positivity was statistically significant in patient group who did not have splenectomy (Table 1). In patient group who had splenectomy, c-mpl was not associated with refractory status.

Table 1. c-mpl positivity in patients group who had and did not have splenectomy.

Summary/Conclusions: Status of c-mpl in ITP is ambiguous. Significant level of positivity in patient group who did not have splenectomy might the useful for splenectomy indication.

PB2111
CLINICAL SIGNIFICANCE OF IMMATURE PLATELET FRACTION MEASUREMENT IN THROMBOCYTOPENIC DISORDERS DURING PREGNANCY
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Background: Thrombocytopenia is the second most common hematologic abnormality during pregnancy and is usually a benign condition. The challenge to the clinician is to weigh the risks of maternal and fetal bleeding complications against the benefits of diagnostic tests and interventions. This condition can also be associated with several diseases, either pregnancy specific or not, such as pre eclampsia, HELLP syndrome, or idiopathic thrombocytopenic purpura (ITP). The differential diagnosis between ITP and gestational thrombocytopenia is clinically important with regard to the fetus, due to the risk of neonatal thrombocytopenia. The immature platelet fraction (IPF) is young cells that have recently been released into the circulation, and are considered indicators of bone marrow recovery. They contain a higher concentration of RNA than mature platelets. Measure of immature platelet fraction (IPF) has been suggested as a less invasive and early diagnostic test in the study of thrombocytopenic disorders. Immature platelet fraction can be currently measured by fully automated hematologaly analyzers providing clinical utility for diagnosing and monitoring thrombocytopenia.

Aims: The aim of this is to know whether IPF can be a useful parameter in pregnant women with thrombocytopenia to predict the potential risk of bleeding.

Methods: Pregnant women with thrombocytopenia were selected (2015-2016); a total of 25 patients (mean age: 33 yrs, range 19-43 yrs) were examined with platelet count <100,000 platelets/μL. Venous whole-blood samples were collected into Vacutainer EDTA-K2E tubes (Becton Dickinson and Company, Plymouth, UK). Complete blood counts and immature platelet fraction (%IPF) were immediately analyzed within 2 h of blood withdrawal by Sysmex XN20 system (Sysmex Corporation, Kobe, Japan). Novel PLT-F channel uses fluorescent light and stains platelets specifically with Oxazine Dye (Fluorescent Fluorocell). Bleeding complication has been collected in order to know if there is related to%IPF.

Results: Mean platelet count was 73,000 platelets/μL (range of 69-91) and IPF mean was 11% (2.5-23.4). Lab test Hemoglobin shows a mean of 95.17 g/L (range of 45-132) (in no-bleeding group was 105.9 g/L whereas in bleeding group was 86.14 g/L, p=0.076) p<0.07. IPF% was <10% in 11, which means a 44% of the patients. 14 patients bleed during or after labor, 56% among all the patients in this study. Related to this group, 11 patients had IPF<10%; 3 of bleeding patients showed an IPF>10%. All pregnant women with an IPF<10% (11/11) bleed as a complication. Pregnant women with thrombocytopenia and an IPF>10% has a higher risk of bleeding during and/or after labor compared with pregnant women with an IPF>10% (Fishier 12.41, P<0.001). 5 (20,83%) patients among all of them were under treatment (earlier or during labor). 3 (12,5%) with steroids and 2 (8,33%) with other methods.
Summary/Conclusions: Thrombocytopenia is a potential risk of bleeding during the labor. A high IPF indicates either consumptive or recovering thrombocytopenic disorders, such as immune thrombocytopenic purpura, while low IPF is characteristic of bone marrow suppression states. Although not directly used in clinical decision making, the reference range is critical to the introduction of new parameters and the interpretation of laboratory results. Our results suggest that the thrombocytopenic parameter analyzed and a level <10% might be an independent bleeding factor which can be useful for detecting high-risk pregnant patients. It should be corroborated in further studies.

PB2112
DOES EARLY RESPONSE TO FIRST LINE CORTICOSTEROID THERAPY PREDICT REQUIREMENT FOR SECOND LINE THERAPY IN IMMUNE THROMBOCYTOPENIC PURPURA?
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Background: Immune thrombocytopenia (ITP) is an acquired, immune-mediated disease that is characterized by increased destruction of platelets by autoantibodies. ITP is characterized by mucocutaneous bleeding. Rarely, life-threatening bleeding such as central nervous system bleeding can occur. Typically, patients have isolated thrombocytopenia. The diagnosis of ITP is one of exclusion. Corticosteroids are chosen as a first-line therapy for adult patients who require treatment. Responses to first line therapy with corticosteroids is about 80% with approximately 20% to 30% long term complete remission. Most patients finally relapse, requiring second-line therapy.

Aim(s): Our aim was to investigate potential effects of early platelet response to corticosteroid therapy on achieving long term complete remission.

Methods: We retrospectively evaluated 43 ITP patients who were followed-up at our institution. All patients’ thromocyte counts were below 30 x10^9/L at diagnosis. All patients received initially methylprednisolone (MP) 1 mg/kg/day. For patients who responded with platelet count ≥150 x10^9/L methylprednisolone was tapered over 3 months. Those who were unresponsive to MP or relapsed after a complete response, were treated with second line therapies that splenectomy or medical treatment agents. The platelet counts of the patients on day 0, day 3, and day 7 were evaluated by complete blood counts and were confirmed with flowcytometry. Early platelet counts on day 3 and day 7 were compared in terms of second line therapy requirement or not. A platelet count of >30x10^9/L on day 3 and >100x10^9/L on day 7 was considered as a complete response. Vaccination against encapsulated organisms was given and imaging was done to detect accessory spleen before splenectomy.

Results: Baseline characteristics of the cohort of 43 patients with an initial diagnosis of ITP are shown in Table 1. The mean age at diagnosis was 51 years (18-84) with female/male: 25/18. All patients presented with severe thrombocytopenia (platelet counts below 30.0 x10^9/L). Most patients presented with mucocutaneous bleeding (n=39), only three patients had genitourinary or gastrointestinal bleeding, and one patient had lymphomatous symptoms. The platelet transfusion aspiration and biopsy was done in 14 (%32.6) patients due to various reasons mainly, failure to respond to ITP treatment (7 patients) and advanced age (7 patients). On third and seventh day of MP therapy, median platelet counts were 30x10^9/L (2.0 x10^9/L - 150 x10^9/L) and 100 x10^9/L (1.0 x10^9/L - 347 x10^9/L), respectively. From baseline to day 7 in each patient, a significant association was found in correlation analysis (p<0.05), 21 patients (%48.8) required second line therapy which were splenectomy (76.2%) or medical treatment (23.8%). Medical therapy consisted of rituximab, eltrombopag, danazol. There was a statistically significant different difference between the patients with platelet count below and over 30x10^9/L on 3rd day of the MP therapy in terms of requirement for a second line therapy, (p=0.04). On the other hand, when 7th day was taken into consideration, there was not a statistically significant different significance when cut off was taken as 100 x10^9/L (p=0.09) or 50 x10^9/L (p=0.08).

Summary/Conclusions: In the era of novel therapies used as second line therapy for ITP, the role of subtypes of T cells in the pathophysiology of ITP and their potential effect on the pathogenesis of ITP can be useful for detecting high-risk pregnant patients. It should be corroborated in further studies.

PB2114
IMMUNE THROMBOCYTOPENIA, EGYPTIAN EXPERIENCE WITH STUDY OF IL-17, TGFB, IL-35 AND IL-12 CYTOKINES IN CHRONIC AND PERSISTENT IMMUNE THROMBOCYTOPENIA PATIENTS
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Background: The role of T cells in the pathophysiology of immune thrombocytopenia (ITP) is heterogeneous and complex. It has been studied in active and reactive ITP but not to same extend in chronic and persistent type.

Aim(s): In this study we review the demographic features of 150 immune thrombocytopenic Egyptian patients and for cases who were chronic and persistent with negative both autoimmune screen and virology for hepatitis B and C.

Methods: We measured IL-12, IL-35, IL-17 and TGFB by ELISA to assess the cytokine secretion pattern in chronic and persistent ITP cases.

Results: Our results revealed Chronic and persistent cases who fulfilled the criteria for cytokine assay were 45 cases with a mean (± SD) age of 31.60±8.78 years. Thirty two patients were presented by skin manifestations (71.1%). Eight patients presented with mucous bleeding (17.8%) and five patients presented with mucous memranous bleeding (11.1%). Comparison between the cases studied and control groups revealed statistically significant differences in level of cytokines. The four measured cytokines were statistically significant higher in cases rather than the control. While the four measured cytokines were statistically significant higher in cases rather than the control. Correlation between platelet count and the level of cytokines was statistically insignificant. All cases were under treatment by low dose corticosteroid in addition to another immunosuppression medication. No correlation between measured cytokines and platelet count.

Summary/Conclusions: This finding of IL-12 and IL-35 is due to significantly higher TH1 activity which explain continuity of the disease while IL-17, IL-35, IL-12 were significantly higher which may explain the pathogenesis of ITP and the disease by effect of immune suppression use or up regulation of their receptors on Treg cells which have resistance to their activity. In chronic ITP, the level of T cell cytokines can’t predict the course of disease.
PB2115
SWITCH OF TPO-MIMETICS IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA: FLORENCE MONOCENTRIC EXPERIENCE
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Background: Primary immune thrombocytopenia (ITP) is an immune-mediated condition characterized by isolated thrombocytopenia, with peripheral blood platelet count <100.000/μl in the absence of an identifiable underlying cause of thrombocytopenia. Clinical studies in patients with ITP demonstrated that thrombopoietin (TPO) mimetics increase platelet production and can outpace platelet destruction.

Aims: We evaluated patients treated with both TPO-mimetics.

Methods: From November 2008 and February 2017, 65 patients were treated with TPO-mimetics with a median follow up of 29 months (1-96); 39 patients underwent therapy with Romiplostim and 26 to Etroltopag. In our study we evaluated 18 patients who received both therapies: among patients treated at first with Romiplostim, 10 patients (9F; 1 M) switched to Etroltopag and 8 patients (3 M; 5 F) switched from Etroltopag to Romiplostim. In the group of 10 patients treated at first with Romiplostim, 5 patients started Etroltopag because were no responders, 3 for loss of response and 2 patients because of adverse events. In the group of 8 patients at first treated with Etroltopag, 4 patients didn’t obtain any response with Etroltopag and switched to Romiplostim, 1 patient underwent to Romiplostim for loss of response and 3 patients because of adverse events.

Results: Among patients switched from Romiplostim to Etroltopag, 2 achieved complete response, 4 response and 4 were no responders; among patients switched from Etroltopag to Romiplostim, 4 obtained complete response, 3 response, 1 no responder.

Summary/Conclusions: Romiplostim and Etroltopag stimulate the TPO-R but have different mechanisms of action, therefore, in our limited experience switching from one thrombopoietic receptoragonist to the other could be beneficial in clinical practice for patients with severe chronic immune thrombocytopenia who failed to respond or experienced adverse events to the first treatment.

PB2116
COEXISTENCE OF GLANZMANN’S THROMbasthenia and MAPLE SYRUP URINE DISEASE: IMPLICATIONS FOR HEMOSTATIC MANAGEMENT
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Background: In Oman, autosomal recessive disorders are relatively commoner than western communities due to the high prevalence of inter-tribal marriage. Unfortunately, some patients have got more than one autosomal recessive genetic disorder, owing to complex consanguinity which might further complicate proper management plans.

Aims: To report an interesting case of combined Glanzmann’s thrombasthenia and MAPLE syndrome, and to review the existing data of platelet function disorders in Oman.

Methods: Case report and retrospective data analysis of all cases with confirmed or suspected platelet function disorders in Pediatric Hematology Unit, Sultan Qaboos University Hospital, Muscat, Oman from January 2006 till December 2016. Results: 38 patients with cerebral aneurysms, scheduled for elective endovascular procedure, were included in the study. All of them had started taking aspirin at a dose of 100 mg daily and cidogrel at a dose of 75 mg daily 7 to 10 days before testing aspirin and cidogrel sensitivity. The following functional tests were performed in all of them before the procedure: 1) VerifyNow® assay: Aspirin Reaction Units (ARU) <550 and P2Y12 Unit Reaction Units (PRU) <200 were considered to be good responses to aspirin and cidogrel respectively. PRU <85 was considered hyper-response to cidogrel. 2) Impedance aggregometry from whole blood (Multplate® analyzer, Roche Diagnostics, Mannheim, Germany): arachidonic acid (AA), adenosine diphosphate (ADP) and thrombin receptor activating peptide (TRAP) were used as agonists. TRAP was used to determine baseline platelet function. Aggregation with AA >10 U and aggregation with ADP >47 U were considered good responses to aspirin and cidogrel respectively. 3) PFA-100: an overall assessment of platelet function was performed using epinephrine-collagen (COL/EPI) and ADP-collagen (COL/ADP) cartridges. Although COL/ADP is not an appropriate method to evaluate the effect of thienopyridines, we performed it to analyze whether hyper-responders to sidogrel detected by VerifyNow® were also identified with PFA-100.

Results: The results of platelet function testing with three different methods are summarized in Table 1. None of the patients showed thrombocytopenia. Good response to aspirin was observed in 84.21%, 97.36% and 93.75% of the patients using VerifyNow®, Multplate® and PFA-100 respectively. Good response to sidogrel was detected in 86.84%, 38.88% and 62.5% of the patients using VerifyNow®, Multplate® and PFA-100 respectively. VerifyNow® identified 6 (13.78%) aspirin-resistant patients. However, PFA-100 and Multplate® showed a significant aspirin-mediated platelet dysfunction in 5 of them. Low response to sidogrel was detected by VerifyNow® in 5 (13.15%) patients consistent with Multplate® results. VerifyNow® identified 10 patients with excessive response, but only 2 of these results were reproduced by Multiplate® or COL/ADP. Multplate® detected 19 patients (50%) with suboptimal response to sidogrel, although these results did not correlate with those obtained by VerifyNow®.

Summary/Conclusions: The effect of aspirin can be accurately measured by platelet aggregation and PFA-100 (with COL/EPI); however, VerifyNow® seems to identify a higher number of poor responders. Multplate® assay using only
ADP is not good enough to detect clopidogrel-mediated platelet dysfunction since it is not specific for the P2Y12 receptor. The addition of GPIIb to the ADP test may increase its sensitivity. VerifyNow® assay seems to overestimate the effect of clopidogrel, since hyper-response data are not reproduced by other techniques. According to our results, a high interindividual variability in response to clopidogrel is observed.

PB2118
THROMBOPOIETIN-RECEPTOR AGONISTS IN ITP - EXPERIENCE OF A CENTER
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Background: Thrombopoietin-receptor agonists (TRA), romiplostim and eltrombopag, are part of the treatment of chronic immune thrombocytopenia (ITP), resistant to first line therapy (corticosteroids and/or immunoglobulins) and with a significant bleeding risk. Both are approved for pediatric use. When used before splenectomy, these treatments may serve as a bridge for surgery or even postpone/avoid the procedure.

Aims: In this report, we aim to evaluate the response to TRA treatment in patients with ITP and associated side effects in our center.

Methods: Inclusion criteria: patients with ITP resistant to first line treatment. Parameters assessed: clinical evolution and adverse effects were evaluated by retrospective analysis.

Results: Thirty-eight patients with ITP were included: 31.4% (12) were male and the median age at diagnosis was 38 years. 44.7% (17) had relapsed/resistant disease after splenectomy and 13.2% (5) were treated with a TRA as a bridge for this procedure. Sixteen (42.1%) of ITP patients were treated with romiplostim: 12 patients (75%) had a response to treatment, and 4 (25%) were resistant. In 11 of these patients, romiplostim was replaced by eltrombopag, either because of resistant disease, or more convenient administration (oral therapy). Thirty-eight (88.6%) patients were treated with eltrombopag (5 pediatric cases); 27 patients (81.8%) responded while 8 patients had resistant disease (3 of these were HIV positive). The response rate was higher in patients with previous splenectomy (91.7% with romiplostim and 92.9% with eltrombopag) compared to those with no previous splenectomy (25% with romiplostim and 73.7% with eltrombopag). Six patients maintained response after treatment suspension (5 treated with eltrombopag and 1 treated with romiplostim). Generally, both treatments were well tolerated, with only one case of eltrombopag suspension because of a thromboembolic event.

Summary/Conclusions: In the current study, both TRA were effective in the treatment of ITP resistant to several lines of treatment, with similar response rates. As described in the literature, the response rate was higher in patients with previous splenectomy, and some cases maintained response after treatment suspension. The toxicity profile was acceptable. However, there are some concerns about their safety in long term therapy, namely the development of myelofibrosis, cytogenetic abnormalities and malignant evolution. Consequently, it is an urgent need for prospective studies to define the optimal period of treatment and surveillance, especially in pediatric patients. In our center, the median time of treatment with eltrombopag for all patients was 5.5 months (range between 1 to 34 months) and with romiplostim was 12 months (range between 1.5 to 85 months). The duration of treatment with eltrombopag in children and adolescents was around 6 months.

PB2119
THE EVALUATION OF REACTIVE OXYGEN SPECIES IN ESSENTIAL THROMBOCYTAEA AND CORRELATION WITH JAK2V617F MUTATION
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Background: Essential thrombocytemia (ET) is a clonal disorder of the hematopoietic stem cells characterized by excessive myeloid proliferation, with predominating megakaryocytic expansion and a potential transformation to acute myeloid leukemia. 50 to 60% of ET cases present a JAK2V617F mutation. 5% to 10% of JAK2V617F negative ET patients have MPL mutations at codon 515 and 50% to 70% of ET patients with non-mutated JAK2 and MPL (double-negative) patients have a mutation at exon 19 of CALR. Genetic instability in ET is associated with an increased level of reactive oxygen species (ROS) which also leads to DNA damage. Hematopoietic stem cells of JAK2V617F positive murine models have higher ROS levels than found in normal mice (Marty et al, 2013).

Methods: We studied 23 patients with ET admitted to the Clinic of Hematology, Filantropia City Hospital, Craiova, Romania, diagnosed with ET according to the 2008 revised WHO criteria (informed consent obtained). All analysis were performed after diagnosis and before the start of therapy. The JAK2V617F mutation was detected by allele specific polymerase chain reaction (PCR) testing. ROS levels were detected by flow-cytometry using a Cy Flow Space Sysmex flow-cytometer and a DCFDA Cellular ROS Detection Assay Kit. Studied parameters were compared both to healthy controls and to each other. Exclusion criteria were patients with secondary condition associated with an increased oxidative stress (alcohol consumption, smoking, diabetes mellitus, hyperlipidemia, chronic renal failure, human immunodeficiency, cirrhosis, and active infection), use of antioxidants or iron supplementation. Data analysis was performed using Flow Max software. The differences between the two groups were assessed using the Student t-test and a p-value of less than 0.05 was considered statistically significant.

Results: The study group involved 12 females and 11 males, with a median age of 48 years. All patients had increased ROS levels at diagnosis compared to healthy controls. Eleven patients had JAK2V617F mutation and twelve were JAK2V617F mutation negative. Significantly higher ROS levels were found in JAK2V617F positive patients compared to JAK2V617F negative patients. The ROS levels were compared both to healthy controls and to each other. Exclusion criteria were patients with secondary condition associated with an increased oxidative stress (alcohol consumption, smoking, diabetes mellitus, hyperlipidemia, chronic renal failure, human immunodeficiency, cirrhosis, and active infection), use of antioxidants or iron supplementation. Data analysis was performed using Flow Max software. The differences between the two groups were assessed using the Student t-test and a p-value of less than 0.05 was considered statistically significant.

Summary/Conclusions: In our study, patients with ET had increased ROS levels. Cases with JAK2V617F mutation associated higher ROS levels compared to those without JAK2V617F mutation. In our future research, we will focus on the follow-up of these patients for a period of four years and we will try to observe if increased ROS levels enhanced genomic instability and transformation to acute myeloid leukemia.
Background: Immune thrombocytopenia (ITP) is an autoimmune disease in children which antibodies develop against platelets (plts) and dysregulation of cellular immunity result in premature destruction of plts and impaired plt production. For most affected children, ITP is a self-limiting disease. Approximately, 10% of all ITP patients eventually develop refractory ITP (RITP). Thrombopoietin receptor agonists (TPO-RA) stimulate thrombopoiesis and are an alternative treatment for RITP. For most affected children, ITP is a self-limiting disease. Approximately, 10% of all ITP patients eventually develop refractory ITP (RITP). Thrombopoietin receptor agonists (TPO-RA) stimulate thrombopoiesis and are an alternative treatment for RITP.

Aims: To evaluate the efficacy and safety of TPO-RA in the treatment of RITP in children.

Methods: Case 1. A 5-year-old girl admitted to the hospital due to ITP with mucocutaneous bleeding. She was refractory to corticoids, immune globulin A. Solé Magdalena1, S. González Muñiz1, M.Á. Fernandez Rodríguez1

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Aims: To estimate the risk of SLE after ITP in adult Jordanian patients.

Background: Pseudothrombocytopenia (pseudoTCP), is incorrectly detection of low platelet counts in automatic blood counter devices and is most frequently caused by ethylene diamine tetra-aeetic acid (EDTA) induced platelet clumping in vitro aggregation. Therefore, pseudoTCP which accounts 15-30 of thrombocytopenic admissions, actually is not associated with a bleeding tendency. PseudoTCP may be an iatrogenic effect of the collection tubes and sometimes the effect can be detected in the EDTA tubes of blood which was collected for another reason.

Aims: To identify the risk factors for pseudoTCP in ITP patients.

Methods: A total of 58 patients (43 females and 15 males) were included in the study. The patients included only those patients who had an initial ANA screen at the time of the diagnosis of ITP. All patients with the diagnosis of ITP were included in the study.

Results: The incidence of ANA positivity was 34% (20/58) of the patients. Among the ANA positive patients, 64% (13/20) had initial positive ANA titer at presentation. The results suggest that patients with initial positive ANA are at risk for development SLE. Thus, follow up after initial positive ANA titer is of great importance as the risk of SLE is significant.

Summary/Conclusions: In our experience, TPO-RA appear to be efficacy and well tolerated in children.

PB2122

INVESTIGATION OF PLATELET FUNCTIONS IN PSEUDOTHROMBOCYTOPENIA

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Background: Pseudothrombocytopenia (pseudoTCP), is incorrectly detection of low platelet counts in automatic blood counter devices and is most frequently caused by ethylene diamine tetra-aeetic acid (EDTA) induced platelet clumping in vitro aggregation. Therefore, pseudoTCP which accounts 15-30 of thrombocytopenic admissions, actually is not associated with a bleeding tendency. PseudoTCP may be an iatrogenic effect of the collection tubes and sometimes the effect can be detected in the EDTA tubes of blood which was collected for another reason.

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Results: The incidence of ANA positivity was 34% (20/58) of the patients. Among the ANA positive patients, 64% (13/20) had initial positive ANA titer at presentation. The results suggest that patients with initial positive ANA are at risk for development SLE. Thus, follow up after initial positive ANA titer is of great importance as the risk of SLE is significant.

Summary/Conclusions: In our experience, TPO-RA appear to be efficacy and well tolerated in children.
Background: The investigation and management of patients with Chronic immune thrombocytopenic purpura (ITP) varies widely. Although many treatments have been recommended for ITP, there are no evidence-based recommendations for when different treatments should be used, or when any treatment should be used rather than managing a patient by observation alone. 

Aims: To evaluate the treatment of ITP patients in Department of Hematology, County Hospital, Timisoara.

Methods: A retrospective study for 350 ITP patients was performed. Patients demographics, medical history, current treatments and side effects, were abstracted from the patient's medical charts for the 15 months prior to their most recent visit.

Results: The mean age was 45.6 years with 58% women and 42% men. Median time from the diagnosis of ITP to the start of the observational period was 23 months. Regardless of the presence of bleeding symptoms, for majority of patients we started treatment based on platelet count. Treatment was considered when platelet counts are less than 20x10^9/L in patients without bleeding, and less than 30x10^9/L in patients with bleeding. Prior to the observational period, 36% of patients had been splenectomized and the most reported treatment was corticosteroids. During the observational period, 72% of all patients were treated. The most frequent reasons given for treatment were platelet count (58%), followed by bleeding symptoms (42%). Corticosteroids represented 52% of treatments, followed by IVig (20%), azathioprine (12%) rituximab and 8% others. Splenectomies (8% of patients) and platelet transfusions (27% of patients) were performed during the observational period. In the patient survey, 52% of participants were 60 years of age or older and the duration of disease was more than 10 years in 43% of patients. The minimum platelet counts were less than 10x10^9/L in 49% of patients. The most common symptoms of ITP was fatigue (45%). Approximately 60% of patients reported at least one side effect associated with ITP treatment. The side effects were most frequently associated with corticosteroid use (43%). Overall, 40% of patients required hospitalization. Mean duration of hospitalization was 13.5 days.

Summary/Conclusions: The retrospective study of 350 patients provides the results of treatment practices in our country. It showed that bleeding symptoms remained quite frequent among patients with chronic ITP. Corticosteroids were the most widely used treatment.

**PB2125**

**IMMUNOLOGICAL THROMBOCYTOPENIC PURPURA AND PREGNANCY: A RETROSPECTIVE STUDY OF 89 PREGNANCIES IN 59 PATIENTS**

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Background: Immunological thrombocytopenic purpura (ITP) occurs for about 1 case for 1000 pregnancies. The risk of onset, aggravation or relapse of ITP during pregnancy is not clearly established.

Aims: The aim is to describe the prevailing ITP progression profile in pregnant women and to evaluate the risk of neonatal thrombocytopenia in two situations, when ITP was known before pregnancy and when ITP was discovered for first time during pregnancy.

Methods: It is a retrospective study carried out in the hematology department of CAC Blida, Algeria, between 1993 and 2016. All patients (pts) who had a pre-pregnancy ITP or thrombocytopenia during pregnancy attached to an ITP were included.

Results: The development of 89 pregnancies (PG), including two twins, occurred in 59 women was analyzed. There were one PG in 40 pts, 2 PG: 13 pts, 3 PG: 5 cases, 4 PG: 1 case and 5 PG: 1 case. Of the 59 pts: in 42 cases it was a history of ITP before pregnancy (group 1: G1) with a history of splenectomy in 9 patients, and in 17 cases it was ITP discovered on the occasion of Pregnancy (group 2: G2). The average age at diagnosis=26.7 years (7-44) and that at delivery=30.4 years (19-44). The mean platelet count at diagnosis: G1: 34000 / µL , G2: 47000 / µL. In the first group (G1): At the beginning of pregnancy the ITP was chronic in 30 cases, newly diagnosed in 1 case, persistent in 2 cases and transient cured in 7 cases; treatments previously received were: corticosteroid therapy (n=34), splenectomy (n=9), Danazol (n=1), cyclophosphamide in 1 case and cyclophosphamide in 1 case, abstention in 7 pts, 2 of whom required corticosteroids during pregnancy. The status of the ITP at the beginning of each pregnancy was: out of treatment (n=8), corticosteroid dependence (n=5), non-response (n=7), PR (n=1), CR (n=24).In the second group (G2): the discovery of thrombocytopenia was in the first trimester (T) in 4 cases, in the second T in 6 cases and in the third T in 7 cases: 17 pts had platelet counts <80000 / µL and were included due to the persistence or even worsening and / or necessity to resort to treatment of thrombocytopenia after delivery. In both groups: in 26 pts (G1:16; G2: 10) variable dose and duration of treatment were required during pregnancy; at delivery, 19 patients needed a treatment, out of them, a bolus of corticosteroids (n=11) transfusion of platelets (n=4), immunoglobulins in 4 cases and transfusion of platelets alone in 4 cases. At birth, thrombocytopenia was observed in 40 pregnancies (50.6%): platelets<30000 / µL (n=7), between 31000 and 50000 /µL (n=13), between 51000 and 100000/µ (n=20), between 100000 and 150000/µl in 2 cases. All pregnancies were completed: 14 by caesarean section, one for thrombocytopenia, with an average platelet count=95000 /µl and 75 by normal delivery with a mean platelet count=100000 /µl with 4 deaths born, one anencephaly and 88 newborns. No hemorrhagic syndrome was observed in pregnancy; two postpartum hemorrhages were seen in G2 group. Eleven newborns (5 in G1 and 6 in G2) were thrombocytopenic with platelet count <20000/µL in 4 cases; between 20000 and 50000/µL in 7 cases; neonatal thrombocytopenia occurred during the first 7 days. Only 4 newborns were treated, one by corticosteroid and 3 by immunoglobulins, with a good progression and only one of the untreated is always followed for thrombocytopenia.

Summary/Conclusions: The de novo ITP appearing during pregnancy is an etiological eventually to be evoked in front of a thrombocytopenia of the pregnant woman after elimination of the other causes related to the pregnancy and in front of the non-resolution after the delivery. The pre-existing ITP does not necessarily.
Quality of life, palliative care, ethics and health economics

PB2126
QUALITY OF LIFE AND SYMPTOM BURDEN IN PATIENTS WITH MULTIPLE MYELOMA
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Background: Multiple myeloma (MM), the second most common hematological cancer, remains incurable. Its incidence is rising due to population ageing. Despite the impact of the disease and its treatment, not much is known about health-related quality of life (QoL) of patients with MM.

Aims: This study aimed to (1) Determine symptom prevalence in patients with MM on disease-modifying treatment, and identify the range and nature of these symptoms within the dimensions of physical, psychological, social well-being. (2) Measure the QoL of patients. (3) Compare the above-mentioned parameters to the general population.

Methods: Adults with multiple myeloma attending the hematology day unit in hematology department from November 2016 to January 2017 were eligible for inclusion in a cross-sectional. Consenting patients completed 2 validated questionnaires: 1) the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 (EORTC QLQ-C30) supplemented by the myeloma-specific module (EORTC QLQ-MY20).

Results: Forty-seven patients were included for analysis: 51%, 1% were male and 48.9% were female. Mean age was 64.7 years (range 42-82, standard deviation 11.0). The QoL scores were significantly lower than the general population (54.7 vs 71.2). The most commonly reported physical symptoms were pain (72%), fatigue (70%) and insomnia (66%). About 61% of the patients were burdened by financial worries. On multivariate analysis, a good performances status (PS≤1) and a response of the disease to therapy (at least a partial response) were associated with high scores of QoL (p<0.01, p=0.03 respectively).

Summary/Conclusions: Patients with MM have a lower QoL than the general population and are symptomatic across physical, psychological and financial domains. They represent a polysymptomatic patient cohort with a complexity of needs that merits a holistic multidisciplinary approach, and consideration of specialist symptomatic or palliative care review.

PB2127
QUALITY OF LIFE IN ANEMIC PATIENTS WITH HEMATOLOGICAL MALIGNANCIES
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Background: Anemia is a common complication of patients with hematological malignancies (HM), which may progress undergoing antitumor treatment significantly decreasing hemoglobin concentration and occur symptoms as fatigue, dizziness, palpitations, dyspnea markedly reduce patient activity, resulting in impaired Quality of Life (QoL).

Aims: To compare of QoL in HM’s patients with different grades of anemia.

Methods: In this study were included following patients (n=326) in the age of 19-82 (Me=65) years: myelodysplastic syndrome (n=37), acute myeloid leukemia (n=20), acute lymphoid leukemia (n=7), primary myelofibrosis (n=23), chronic myeloid leukemia in blast crisis (n=6), multiple myeloma in II and III st. (n=126). Non-Hodgkin’s lymphoma in III-IV st. (n=40) and chronic lymphocytic leukemia in B or C st. (n=67). Patients were examined: 1) clinical blood test (hemoglobin concentration) to assess anemia’s grade; 2) the Functional Assessment of Cancer Therapy-Anemia (FACT-An) scale to measure of QoL. The FACT-An questionnaire consists of a general questionnaire (FACT-G), measuring domains of physical, social/family, role (SR), and thus the focus of our analysis. The American paper incorporates outcomes from 6 RCTs for treatment with LMWH and the CAT medication 11% of the total cost of CAT. The annual medication costs of LMWH for daily treatment in 365 days were 32,120 USD in wholesaler purchase strategy (WPS). For VKA the annual medication cost for 365 days was 44 USD. LMWH is the cost driver but is not cost effective due to the cost of the study. The finding is that “The one-way sensitivity analysis shows that LMWH would become the preferred strategy once its annual cost was less than $7177.” In the present analysis, the daily cost acquisition cost Wholesaler Purchasing Price (WPP) (which corresponds to the American WPS) and the cost driver in the newest and most relevant health economic research and compared it with the costs from 6 European countries as well as Canada.

Aims: To highlight the importance of localized or regionalized cost inputs as cost drivers when considering cost effectiveness in relation to CAT.

Results: Simply by applying the local unit cost for the treatment with LMWH for these countries, the conclusion becomes notably different. LMWH becomes the cost effective alternative in European countries as well as in Canada with annual costs below 7177 USD. The price for VKA is comparable to that in the US, and does not change the cost effectiveness ratio. The data from the retrospective cost of CAT study that the cost of the hospitalization of 19% of the total cost of CAT and the CAT medication 19% of the total cost of CAT. This outlines hospitalization is a cost driver as well and not only the medication. Similar conclusions were reached in other studies. In summary, the role of the cost driver can change as a consequence of the localization of the costs. This outlines the great variation in costs in terms of CAT, and the caution it must be used with (Table 1).

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Table 1.
MINIMIZING THE RISK OF MUCOSITIS IN HEMATOLOGICAL PATIENTS WITH TOPICAL PRODUCTS

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Background: Mucositis is a frequent severe complication associated to aggressive therapies of hematological malignancies with chemotherapy and/or radiotherapy, conditioning therapy in stem cell transplants. Regularly occurs at 3 to 10 days after chemotherapy and about 6 to 8 weeks after radiotherapy. It is self-limited within 2-4 weeks, but in this period the patient is vulnerable to systemic infections (bacterial and fungal). It could also compromise the optimal timing and dosage of the chemotherapy schedule, induce psychosocial distress, prolonged hospitalization and finally, higher costs.

Aims: Evaluating the efficacy of GelX® in chemotherapy induced mucositis. GelX® is a topical product that contains Zinc gluconate+taurine, with bacteriostatic and anti-inflammatory effect, easy to use for the patient, in order to prevent and reduce pain and severity of oral ulcers, making a barrier for mucositis.

Methods: A retrospective analysis of 77 adult patients: 17 with hematological treatments and 60 with algogenic stem cell transplantation. 17 were diagnosed and treated between January 2016 and December 2016 with various hematological malignancies (5 AML, 2 ALL - 1 Ph positive, 2 BLastic phases of CML, 3 AILT, 1 ATLL, 2 MMM, 2 CML, 1 MDS, 1 BH), 16 cases of grade 3-4 mucositis has appeared. The conditioning regimen was mieloablative (3 cases), and reduced intensity (21 cases). There were 21 cases of sibling allotransplants (GAMIL, 3 ALL, 1 ATLL, 5 LMNH, 1 CLL, 2 SAA, 2 CML, 1 MM) with 10 cases of mucositis grade 3-4. The regimens used were 6 mieloablative and 15 nonmieloablative. 3 from 4 cases of haplotransplant with nonmieloablative conditioning (2MDS, 1 AAML and 1 SAA) had grade 3 mucositis.

Results: GelX® induced a reduction in the grading of mucositis (grade 1-2) and a shorter period of evolution (5 days) versus grade 3-4 mucositis and prolonged duration of oral lesions for those with curative treatment. From 60 patients allotransplanted, 30 patients experienced grade 3 and 4 mucositis with a medium duration of five days. All of them received GelX® as prophylactic treatment.

Summary/Conclusions: Prophylaxis is the key of successful evolution in mucositis (time to heal shorter than 10 days). Identifying candidates for mucositis is mandatory and the product should be applied starting with the chemotherapy (or in the first 24 hours on the onset of chemotherapy) in order to minimize the risk of mucositis appearance.

EUROBLOODNET: THE EUROPEAN REFERENCE NETWORK IN RARE HEMATOLOGICAL DISEASES

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Background: Under diagnosis related to the earlier hemoglobin (Hb) or hematocrit (Hct) diagnostic criterion is one reason to the 2016 revision of the diagnosis of PV in the World Health Organization (WHO) classification of Tumours of Haematopoietic and Lymphoid Tissues. Bone Marrow Biopsy (BM) and molecular markers (JAK2) are recommended to establish the diagnosis in those with the lower threshold(Arber DA et al.2016). This potentially could result in increased numbers and costs of investigations. The lower thresholds are aimed to identify those previously referred to as masked PV (mPV) who have been recognized to have an increased incidence of thrombosis (Barbui T et al, 2014 & 2015).We hypothesized that the revision would increase the incidence of patients with thrombosis, stroke and potential PV who would then require additional investigations.

Aims: To determine number of patients with young strokes with potential PV on application of the 2016 revised WHO criteria for PV.

Methods: We undertook an analysis of records of patients with ischemic stroke treated by us mainly maintained in the 11th Indo-US Stroke Registry and Infrastructure Development Project. This registry enrolled adult patients admitted with imaging-confirmed ischemic stroke <2 weeks after symptom onset. The Indo-US Stroke Registry and Infrastructure Development Project, includes 5 geographically diverse centers in India and one in USA. The registry data was entered into a well-based electronic database. From January, 2012 to March, 2014, 2076 patients with new onset ischemic stroke were evaluable in the Indian arm of the Indo-US Stroke Registry. We compared the incidence of polycythemia as per the 2016 revision against the earlier (2008) Hb diagnostic criterion.

Results: There were 24 (1.2%) patients with potential PV which was revised to 111 (5.3%) on applying the 2016 Hb criterion. The McNemar test determined that there was a statistically significant difference in the proportion of polycythemics, p= 0.000. Considering the potential of comorbidities in the elderly to confound the association of polycythemia with ischemic stroke, we...
separately analyzed only those with young stroke (Age <45). In this cohort there were 420 patients. A total of 6 (1.4%) patients had potential PV based on the 2008 Hb criteria. On applying the 2016 revision; 37 (8.8%) patients fulfilled the Hb criteria. An exact McNemar’s test determined that there was a statistically significant difference in the proportion of polycythemes, p= 0.000. Separate analyses by gender was not significant in females, P=0.5; but significant in males, P<0.05. The incidence of the 4 cases of splenomegaly was analyzed with the revised criteria for polycythemia. The impact of cost in influencing treatment decision from resource limited countries with predominant out of pocket health expenditure has been earlier reported (Phillip C et al, 2015). This revision promotes the routine use of BM and JAK-2. In our analysis we estimate this new criterion would add to the costs to each patient (~ 7000 per centre estimate).

Summary/Conclusions: The present data shows that there exists a significant difference in the incidence of polycythemia in thrombosis (Ischaemic Stroke) on applying the revised criteria. The requirement to additionally investigate them with BM and molecular markers for PV has potential economic implications.

PB2132
PATHOPHYSIOLOGICAL MECHANISMS INVOLVED IN THE DEVELOPMENT OF ANEMIA IN PATIENTS WITH NON-HODGKIN’S LYMPHOMA
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Background: Non-Hodgkin’s lymphomas (NHL) are a group of heterogeneous malignant lymphoid disorders that associate anemia either from diagnosis or during the evolution of the disease. The anemic syndrome can be present at the moment of diagnosis or can develop during the evolution of non-Hodgkin’s lymphomas, with frequent involvement of the hematopoietic system due to interference of some anemia-producing factors, such as age, gender, smoking, and disease-related factors. Various pathophysiological mechanisms responsible for the development of anemia are described in literature: pro-inflammatory cytokines and hepcidin action on iron metabolism and erythropoiesis, bone marrow failure caused by infiltration of malignant lymphocytes, cytokines, and hepcidin action on iron metabolism and erythropoiesis, bone marrow failure caused by infiltration of malignant lymphocytes, cytokines, and very aggressive lymphomas in 26%. NHL repartition on stage of disease revealed: type I – 2.35%, type II – 18.81%, type III – 57.64%, and type IV – 23.36%.

Results: In our study group, the median age at diagnosis of non-Hodgkin’s lymphoma was 64 years, sex distribution was males:females=1.3, and the rural to urban area index=1.2. 85.88% of patients had B type NHL and 14.12% T type NHL. 20% of NHL were indolent lymphomas, aggressive lymphomas in 54% cases and further stages of NHL were revealed: type I – 2 – 3.25%, type II – 18.81%, type III – 57.64%, and type IV – 21.16%. In our study group, 84% of patients enrolled had anemia, with the anemic syndrome affecting the 50-59 years and 70-79 years age groups. 59.73% of patients had anemia at diagnosis and 40.27% of patients developed anemia during the evolution of NHL. The pathophysiological mechanisms involved in the development of anemia were: perturbations of iron metabolism and erythropoiesis under pro-inflammatory cytokines and hepcidin action (47.2%), bone marrow failure induced by lymphomatous infiltration (25%), anemia induced by chemotherapy (18.05%), and autoimmune hemolysis (9.7%). Five patients with anemia induced by chemotherapy and three patients with lymphomatous infiltration of the bone marrow also associated iron and/or folate deficiency.

Summary/Conclusions: In our study, anemia was present in 84% of NHL cases, more frequently found in patients that associated comorbidities and belonged to the 50-60 years and 70-80 years age group. In half of the cases, anemia was moderately severe. 47.25% of patients had simple chronic anemia due to perturbations of the iron metabolism and of erythropoiesis, and 25% of patients presented anemia due to bone marrow failure. Chemotherapy seemed to lead to an anemic syndrome in 18.05% of cases, whereas hemolysis of autoimmune causality was diagnosed in 9.7% of cases. Differently diagnosed the management of anemia is extremely important in patients with NHL because it influences the administration of chemotherapy (dose intensity and density), prognosis and quality of life.

PB2133
HEMATOLOGIA, CENTRO MÉDICO HEMATOLÓGICO, 2LABORATORIO ELEA, BUENOS AIRES, ARGENTINA, 3AMBEXIENCE, MADRID, SPAIN

Background: Novex® is a biosimilar by design of the reference product Mabthera®/Rituxan®. Novex® was approved in Argentina following ANMAT’s Biosimilar guidelines, having the same indications as the reference product, Rituxan. The development of Novex® is commercialized by Laboratorio ELEA. As part of its Risk Management Plan (RMP), Laboratorio ELEA implements an active pharmacovigilance program as defined in Argentina regulation. Periodically reports ANMAT RMP status and results.

Aims: To describe frequency and pattern of adverse events during the use of Novex® in treatments registered along an active pharmacovigilance program in order to oversee the safety profile of NOVEX® in the real clinical practice and maintain the benefit-risk evaluation.

Methods: A treatment Registry for NOVEX® was implemented from the beginning of NOVEX® commercialization as part of the RMP. The Data Lock Point for this report is Jan 31st 2017. Physicians prescribing NOVEX® were request- ed to fill a form indicating age and gender, treatment start date, treated pathol- ogy, dosing and dose frequency. Such data was recorded in a database. After a preset time, physicians were contacted by Laboratorio ELEA to ask them about the treatment outcome and Adverse Event occurrences. If adverse events were detected they reported each occurrence as Individual Case Safety Report (ICSR), were they were registered using the MedDRA dictionary (version 19.1) for its coding.

Results: The total number of participating physicians was 151. During this period, they reported 638 treatment initiations, 389 of which had at least 1 follow up point and were included in further analysis. 53% male. Mean age 64.1 years. Anana- tological indications were more than 90%. More than 90% of indications were approved indications. Nevertheless, we detected off-label use. Total cycles received for any approved indication had a mean number of 5.7. Total received Individual Case Safety Reports were 17, indicating a relative frequency of 4.4% for this phase of the case Safety Report. Occurrence rates were 0.02 per 100 administered cycles, and 0.020 per 100 treatment days. Eleven Individual Case Safety Reports were classified as serious (SAE) because they had at least one manifestation that prolonged hospitalization, endangered life or was death-associated. The most frequent AE reported was acute reaction related to infusion (9 cases), followed by cardiovascular manifestations (2 arrhyth- mia, 1 cardiac failure and 1 ischemic stroke), infections (1 pneumonia, 1 pro- gressive multifocal leukoencephalopathy), neurologic (1 paresthesia), cytopenias (1 pancytopenia) and cutaneous (1 bullous dermatitis).

Summary/Conclusions: The activities developed under this active pharma- covigilance program showed great value allowing us not only to monitor the adverse event pattern but also to detect off-label use as part of real life treatments. This report showed a similar safety profile to that of the reference product concluding that NOVEX®, in terms of tolerability, is similar to the reference product. Pharmacovigilance is cornerstone in the development of biologics, especially biosimilars, as a tool to assist in the knowledge about their safety profile.

PB2134
DEPRESSION AS THE PRESENTING SYMPTOM OF CENTRAL NERVOUS SYSTEM LYMPHOMAS IN NORTHWESTERN TURKEY
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Background: PCNSL represents approximately 4 percent of newly diagnosed primary central nervous system (CNS) tumors, with an age-adjusted incidence rate of four cases per million persons per year. Most cases of non-AIDS related PCNSL are diagnosed in patients between 45 and 65 years of age, with a median age at diagnosis in the fifth decade. The most notable risk factor for the development of PCNSL is immunodeficiency including HIV infection, iatrogenic immune suppression, and congenital immune deficiencies. Antecedent flu-like or unusual febrile illness was reported in 9.7% of patients. Treatment-related complications and very aggressive lymphomas were reported. Presenting symptoms may include focal neurologic deficits, neurophysiologic symptoms, signs of increased intracranial pressure, seizures or ocular symptoms. Neuropsychiatric symptoms like depression, apathy, psychosis, confusion, memory impairment, slowness of thought are generally undernoticed or overdiagnosed due to the lack of recognition of the underlying disease. Physicians not recognizing these symptoms may be negatively biased towards antidepressant use. Diagnosis is based on imaging of the central nervous system (CNS), ideally with contrast-enhanced magnetic resonance imaging (MRI), cerebrospinal fluid (CSF) analysis, unless contraindicated due to elevated intracranial pressure. The radiographic lesion tends to be a solitary non-hem- oangioma mass, situated in the deep white matter adjacent to the ventricular surface.

Aims: We aimed to evaluate the presence of depression and antidepressant use before the diagnosis of CNS lymphoma and emphasize the duration between the diagnosis of depression and lymphoma. Methods: Data on 40 patients with CNS lymphomas were evaluated in a retro- spective manner. From their national health records, prescription for antidepressant and anxiolytic drugs with their psychiatric diagnosis, time before the diagnosis of CNS lymphoma, the branch of the prescribing physician, presenting symptoms

haematologica | 2017; 102(s2) | 845

Madrid, Spain, June 22 – 25, 2017
from their medical files, type and treatment of lymphoma and survival were recorded. OECD international statistics as well as Turkish Statistical Institute data for national antidepressant use were collected and interpreted.

Results: Of the 40 patients, 14 were male (35%) while 26 were female (65%). Mean age was 60.5 years (38-78), 7 patients were alive (17.5%). Method for diagnosis was radiological imaging (magnetic resonance imaging) in 27 patients (67.5%) while in 13 patients, diagnosis was supported with histopathological confirmation (32.5%). Mean survival was 8.6 months (2-24 months). As the complaint for medical help seeking, 4 patients presented with neurophysiologic symptoms while 16 patients presented with headache (40%) and 20 patients (50%) presented with neurologic deficits. On the other hand, prior to lymphoma diagnosis, 7 patients were diagnosed as anxiety disorder and 13 as depression (total, 19 patients, 47.5%) and were prescribed antidepressant and anxiolytic medications. The mean duration between prescription of antidepressants and diagnosis of lymphoma was 2.6 months (0-10 months). Within the patients who were on antidepressants, 6 were female and 14 were male.

Methods: An economic model was developed to estimate the incremental total healthcare costs by year are considered in the model were drug, drug administration, adverse events (AE), AE monitoring, one-time progression and terminal care costs. The role of laparoscopic splenectomy (LS) in patients with hematologic malignancies is still unclear. Nevertheless, the ageing of the world’s population and the increased incidence of Non-Hodgkin’s Lymphoma are increasing the indications for splenectomy, requiring a well-tolerated and less invasive procedure.

Aim: The aim of this review is to analyze our single-center experience of LS performed for malignant Hemopathies. Results are compared with LS for benign splenomegaly and the risk of locoregional dissemination or inadequacy of fragmented histological sample were analyzed.

Methods: We retrospectively analyzed 50 patients who underwent LS between 2005 and 2016 at Saint-Pierre Hospital. Among these, 12 patients were on antidepressants, 6 were female and 14 were male. The mean duration between prescription of antidepressants and diagnosis of lymphoma was 2.6 months (0-10 months). A significant difference in terms of platelets recovery after 1 month from the surgery was shown in patients efficiently Vs inefficiently operated (respectively 387 +/- 125 Vs 138 +/- 90 x 10^3/mL, p<0.05). The median follow up is 39 +/- 37 months and 80% achieved a hematological recovery.

Summary/Conclusions: LS is a safe and less-invasive procedure in patients affected by Malignant Hemopathies. This approach is also well tolerated in older patients (median 67yrs) and in patients with large spleen (1515 +/- 660 mL), extending the indication for laparoscopic SHM even in older patient and in patients with high volume spleen. Compared to historical data, LSy for Malignant Hemopathies shows better early and late morbidities. Our data shows however a trend for higher late morbidity in the SMH group, warranting a careful long term follow-up in this subset of patients.
patient due to urgent admission, long duration of stay in hospital, chemotherapy agents used in the treatment and the disease itself. Evaluating this group of patients for anxiety and depression, providing necessary professional support and revising medical treatment is therefore substantial.

**Aims:** In our study, we aimed to assess the risks of anxiety and depression in newly diagnosed acute leukemia patients who were admitted to hematology clinic to receive chemotherapy and provide necessary professional support along with treatment revisions and follow-up according to our findings.

**Methods:** Our study was performed with newly diagnosed acute leukemia patients, who were admitted to our hospital hematology clinic in a six-month period to receive chemotherapy. Demographic characteristics were noted and Hospital Anxiety and Depression Scale (HADS) was used to assess depression. Hospital Anxiety and Depression Scale (HADS) is an assessment scale developed by Zigmond and Snait to determine the risks and assess the severity of anxiety and depression (8). The validation and reliability studies of the scale in Turkey were carried out by Aydemir et al (9). The questionnaire has a total of 14 items: seven of which measure anxiety (odd numbers) and the remaining seven (even numbers) measure depression. Each item is scored from 0 to 3. The scoring order of each item in the questionnaire is different. Items numbered 1, 3, 5, 6, 8, 10, 11 and 13 indicate decreasing severity and are scored as 3-2-1-0. On the other hand, items numbered 2, 4, 7, 9, 12 and 14 indicate increasing severity and are scored as 0-1-2-3. The cut-off value for the total score of the odd-numbered questions assessing anxiety is 10; while it is 7 for the even-numbered questions assessing depression.

**Results:** 21 patients were included in the study. 13 of these patients (61.9%) were diagnosed with acute myeloid leukemia (AML) and 8 (38.1%) were diagnosed with acute lymphoblastic leukemia (ALL). Median age of the patients was 45 (range: 21-69). 11 patients (52.4%) were female and 10 (47.6%) were male. 5 patients (23.8%) had comorbidities while 16 (76.2%) had none. Anxiety evaluation revealed 38.1% of all patients in the study experienced anxiety. The rate of anxiety was 38.5% in AML patients and similarly 37.5% in ALL patients. 45.5% of the female patients had anxiety while the rate was only 30% in male patients. The difference was not statistically significant (p >0.05). Depression evaluation revealed that 81% of all patients in the study. The rate of depression was 84.6% in AML patients and 75% in ALL patients. 81.8% of the female patients had depression while it was 80% in male patients. Neither anxiety nor depression had a significant correlation with comorbidities or gender (p >0.05). Correlation analysis revealed a positive correlation between anxiety and depression (r=0.846; p <0.01).

**Summary/Conclusions:** In conclusion, assessing anxiety and depression in patients with acute leukemia is a part of the course of and adherence to treatment. In our study, depression was distinctly more common than anxiety and there was a positive correlation between depression and anxiety. We think that including a professional for psychosocial support in the medical team is important for the treatment of these patients.

**Background:** Three years ago, a unit for autologous bone marrow transplant for hematological patients has been established in Shaare Zedek medical center. The patients meet with the doctors for the treatment plan usually following the diagnosis. From the point of view of a part of the patients, the process appears simple, short term, and promises cure. In reality, the process is long term, including aggressive chemotherapy prior to the transplant. The treatment is highly aggressive and toxic with many physical and mental side effects for the patient and his/her family. The transplant process requires hospital admission for about a month in an isolation room. No one is allowed in the room except for close relatives and the medical staff. The social worker, part of the caring staff, accompanies patients and families from the initial diagnosis through this taxing and stressful process. Most patients are young, average 45 years, in the middle of their careers, from a broad spectrum of occupations, education as well as social status, representing Israeli society.

**Aims:** To accompany and empower patients by means of giving them tools to cope with the transplantation process which is a crisis situation in the midst of their lives. 2. To teach patients self-awareness. 3. Promote quality of life for the patients especially during the stay in the isolation room by way of creating a safe domain.

**Methods:** The following tools had been utilized: 1. The “Empowerment method”. An advanced view of the powers and experiences of patients that constitute resources in addressing crisis. 2. Work of hope- finding unique meaning in life crisis.

**Results:** This work is based on therapeutic conversations that took place inside the isolation room with about 30 patients, mostly men, average age was 50, during the past three years. With the understanding that a patient goes from the public sphere to a private one -the isolation room- my entrance into the room was based on the ability and willingness of the patients to go into a treatment dialogue at that point and time. From the narratives of the patients, a few themes were extracted that were repeatedly discussed by most patients. 1. Fear of death 2. Post-treatment issues 3. Fear of isolation 4. The issue of relationships. 5. Mind and body. 6. Children. 7. Faith. 8. Closure

**Summary/Conclusions:** From the therapy sessions it appears that the central issue for the patients during the isolation period is the struggle with the fear of death. The process of treatment helps patients to go from the private sphere back to the public one.

**Recommendations:** It seems essential for the patients in the isolation room, undergoing autologous bone marrow transplant, to have therapy sessions with a qualified social worker as part of the holistic care. ‘Having a room of his own’ in the process enables an opportunity to examine the inner self esteem and strengths of the patients thereby patients learn to contribute to themselves from themselves.

**PB2138**

**GENDER DIFFERENCE IN ANXIETY FOR THE FIRST BLOOD TRANSFUSION**

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**Background:** Blood transfusion has several risks including allergic reaction, acute hemolysis, infectious disease and so on. Both physicians and patients are always cautious to decide on blood transfusion.

**Aims:** The purpose of this study was to explore whether there are gender differences in anxiety for the first blood transfusion in patients with different diseases.

**Methods:** 315 patients (153 men and 162 women ) were enrolled in this prospective, comparative study and median age was 38 years(range 17-72). The disease consisted of 85 chronic hepatitis B, 73 leukemia, 69 gastric ulcer, 48 chronic renal failure and 40 gynecological oncology. Various blood products including plasma, red blood cells suspension and platelet were infused. Anxiety was evaluated according to the HAMA self-rating anxiety scale (SAS) during the first blood transfusion. Patients got 50 points below were divided into no anxiety group, 50 to 59 points were divided into mild anxiety group, 60-69 points were divided into moderate anxiety group and 70 points or more were divided into severe anxiety group.

**Results:** For patients with the same disease, more female patients were divided into moderate to severe anxiety group than male ones. The number of patients with mild anxiety was similar in female and male, and no one was divided into no anxiety group.

**Summary/Conclusions:** Women were more anxious than men during the first blood transfusion, which is independent of age, race, education level and kinds of blood product.
Sickle cell disease

PB2140

HYDROXYUREA INHIBITS MYELOID DIFFERENTIATION VIA NITRIC OXIDE SYNTHASE

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Background: Hydroxyurea and nitric oxide (NO) inhibit erythroid differentiation, while hydroxyurea is NO-releasing agent used in therapy of sickle cell diseases in children and adolescents. Aims: To study the mechanism of hydroxyurea inhibition of erythroid differentiation by exploring NO synthase (NOS) dependence. Methods: The erythroid differentiation is studied by methylcellulose colony assay in mice, whereas presence and activation of endothelial NOS (eNOS) by immunocytochemistry and immunoblotting, respectively in K562 erythrocytic cell line. Results: In ex vivo experiments, mice exposed 7 days to hydroxyurea demonstrated significant decrease in the number of nucleated cells per femur, partially reversed by NOS inhibitor N-nitro L-arginine methyl hydrochloride (L-NAME). The results point out that NO is involved as a mediator, but the prominent reduction of colons has been observed with NO metabolites nitrite (NO2) and nitrate (NO3). Moreover, hydroxyurea demonstrated a large diminution in the number of bone marrow derived myeloid colony-forming unit-granulocyte/macrophage (CFU-GM), burst-forming-units-erythroid (BFU-E) and colony-forming unit-erythroid (CFU-E) colonies in methylcellulose cultures. L-NAME attenuated hydroxyurea reduction of myeloid and erythroid colonies, while it itself increased CFU-E and CFU-GM colonies and slightly BFU-E colonies. NO metabolites NO2 and NO3 generally inhibited myeloid and erythroid colonies, but the reduction was more prominent by NO2 compound. Moreover, the hematological parameters weight (before and after treatment) of mice did not show any significant difference among studied groups. Hydroxyurea increased NO production and the number of eNOS positive K562 erythrocytic cells, while phosphorylation of eNOS and activation of AKT/mTOR signaling was not blocked by phosphatidylinositol 3-kinase inhibition. Summary/Conclusions: NO produrg hydroxyurea demonstrated NOS dependence in inhibition of myeloid / erythroid differentiation, not influencing the hematological parameters.

PB2141

SLEEP DISORDERED BREATHING IN CHILDREN AND ADOLESCENT WITH SICKLE CELL DISEASE: IMPACT ON EXECUTIVE FUNCTION AND PROCESSING SPEED INDEX

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Background: Studies in non-syndromic children have shown that sleep-disordered breathing (SDB) increases the risk of neuropsychological deficits and neuronal brain injury. Few authors have investigated the role in cognitive deficits of SDB and the associated hypoxia in children with sickle cell disease (SCD). Snoring and SDB are very common in children with SCD and may affect cognitive function in very young children. Previous data suggested that executive function was worse in older children with SCD and low and mild mean overnight oxygen saturation. Aims: We aim to investigate if SDB could be a potential factor contributing to developmental problems in cognition in children and adolescent with SCD. Methods: We have followed up children and adolescents in the Sleep Asthma cohort who underwent Polysomnography at two different time points (1) 2006-2009 and (2) 2011-2014 and compared the sleep data with subsequent neuropsychological assessment. Results: Worse performance was found for processing speed: PSI (p<0.01) and general intelligence (p<0.05) compared to controls. SDB, measured as apnea and hypoxia index (i.e. AHI >3%): Apnoeas and hypopnoeas with more than ≥3% desaturation, was found to impact executive function, as assessed with the Tower test. (p<0.05) and PSI (p<0.05). Mean oxygen saturation during total sleep time was significantly associated with lower PSI (p<0.05). Additionally, participants who showed a worsening of their SDB symptoms in their second sleep study had lower cognitive scores (i.e., executive function, p<0.05 and PSI, p<0.01) (Figure 1). Summary/Conclusions: SDB symptoms seem to worsen into adolescence and therefore, might have a neurodevelopmental impact if left untreated; appropriate intervention might improve cognition and quality of life.

PB2142

LUNG FUNCTION IN CHILDREN AND ADOLESCENTS WITH SICKLE CELL ANEMIA: A COMPARISON BETWEEN UK AND ITALY

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Background: Acute and chronic respiratory complications are common in sickle cell anemia (SCA). Subjects with SCA often have a progressive decline of lung function with age that could be influenced by the quality of healthcare and by environmental factors, such as the level of exposure to air pollution. Aims: To compare lung function, evaluated cross-sectionally through spirometry, in children and adolescents attending sickle cell centers in UK and Italy. Methods: Anthropometry and spirometry were recorded in patients with SCA (SS,SB2) aged 6-17 years of African ancestry followed at the Evelina Children’s Hospital, London, UK, and at the University Hospitals of Padova and Udine, northeast of Italy. Subjects from the British cohort lived in an urban area while those from Italy came from urban and non-urban areas. Exclusion criteria were the presence of SCA-related morbidity within the last two weeks and the inability to perform a spirometry meeting the European Respiratory Society acceptability and interpretability criteria (Miller, Eur Respir J 2005;26:319–338), modified for children (Kirkby, Pediatr Pulmonol 2008:43:1233–1241). Portable spirometers (Pony FX, Cosmed-IT, Easy-on PC, NDD-CH) were used. Z-scores of anthropometric and spirometric data were derived, respectively, from CDC2000 and from the Global Lung Initiative 2012 predictive equations for African Americans (Quanjer, Eur Respir J 2012: 40:1324–1343). Spirometry patterns were classified as normal, obstructive (zFEV1/zFVC<1.64) or restrictive (zFVC<1.64+zFEV1/zFVC ≥ -1.64). Differences between groups were assessed by t-tests and considered statistically significant for p values <0.05. Results: A total of 101 children and adolescents were included (n. 62 in UK; n. 39 in Italy; 42% girls; age-range: 6.2-17.9 years). We didn’t find significant differences in mean spirometry indices between the SCA cohort from London and northeast Italy (Table 1). Nevertheless while an obstructive spirometry pattern was more common in the British cohort compared to the Italian one (respectively 22.5% vs 7.7%), the picture was the opposite for the restrictive pattern (respectively 11.2% and 20.5%) (Table 1). In the whole sample age was negatively correlated with both zFEV1 (Spearman’s rho-0.20) and zFVC (Spearman’s rho-0.24).

Table 1.

<table>
<thead>
<tr>
<th>Index</th>
<th>Sickle cell UK</th>
<th>Sickle cell FTA</th>
<th>Diff between means (5%) CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>zFEV1</td>
<td>0.55 (0.05)</td>
<td>0.61 (0.05)</td>
<td>0.06 (0.01 to 0.10)</td>
</tr>
<tr>
<td>zFVC</td>
<td>-0.11 (-0.23)</td>
<td>-0.08 (-0.19)</td>
<td>0.03 (0.01 to 0.05)</td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>0.55 (0.11)</td>
<td>0.54 (0.11)</td>
<td>0.01 (0.00 to 0.02)</td>
</tr>
<tr>
<td>zFEV1</td>
<td>-0.10 (-0.24)</td>
<td>-0.09 (-0.24)</td>
<td>0.01 (0.00 to 0.02)</td>
</tr>
<tr>
<td>zFVC</td>
<td>-0.11 (-0.24)</td>
<td>-0.11 (-0.24)</td>
<td>0.00 (0.00 to 0.01)</td>
</tr>
</tbody>
</table>

Summary/Conclusions: Lung function of pediatric subjects with SCA living in London and in the northeast of Italy is overall comparable. Obstructive lung disease is more common among subjects with SCA living in London than in urban and non-urban areas in Italy. Differences in the level of exposure to ambient air pollution and in the prevalence of allergies between the rural and urban environment might have contributed to this finding and need to be further investigated.

PB2143

SICKLE CELL DISEASE: A NEW DISEASE IN MADRID

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Background: Sickle cell disease (SCD) was scarcely diagnosed 2 decades ago in Spain, and the Community of Madrid is a paradigm of the adjustments that had to be implemented to attend an increase of cases due to immigration. Aims: The aim of our study was to find out the prevalence of SCD in the referral sickle cell newborn screening of the Community of Madrid, in addition to the demographic characteristics of these patients. The secondary objectives were to obtain the frequency of specific treatments or prophylaxis accomplished by these patients, and the reasons for loss to follow-up.

848 | haematologica | 2017; 102(s2)
Methods: The study was observational, unicentric, descriptive and retrospective, carried out in February 2017 in a tertiary hospital in the Community of Madrid, Spain. All patients diagnosed with SCD and who had attended at least once to the hematology clinic for this reason were included. Demographic characteristics (date of birth, gender, country of birth) and clinical characteristics (genotype, therapy and update in follow up, like alive, deceased or lost patient) were collected. Written informed consent was signed by patients or legal guardians in accordance with the Declaration of Helsinki. The study was approved by the hospital Ethical Committee. Statistical analyses were performed using SPSS version 18.0. Quantitative variables were reported as median or mean value and range, while categorical variables were expressed as absolute value and percentage.

Results: The total number of SCD patients included was 209. Ratio boy/girl is 1.3. Most of patients were born in Spain (85%), although 8% and 5.26% were born in Africa or America respectively. Seventy three percent of the progenitors came from Africa and 24% from America. Ninety two percent of those SCD patients born in Spain were detected in the first days of life due to universal screening detection implemented in Community of Madrid since 2003. Median age at first diagnosis was 1.42 years (0-21.4). Median age at the end of inclusion was 9.91 years (range 0.13 to 35.14). SS or S/Betathal was reported in 86%. In addition, 2.39% associated alfa gen deletion, and 1 (0.48%) glucose 6 phosphate dehydrogenase deficiency. No patient had congenital thalassemia. Eighteen patients (8.65%) had human leucocyte antigen (HLA) identical siblings. Hydroxyurea was added to standard treatment in 65 patients (31%) of which 47 continue to be treated to date. Penicillin prophylaxis was communicated in 165 patients (79%). Vitamin-D prophylaxis was initiated in 128 patients (56%). Other changes in treatment and clinical issues included 21 cases (12%) and 9 children (4%) underwent splenectomy. None of these patients had sepsis or meningitis. Cholecystectomy was performed in 9 cases (4%). There were 18 progenitor stem cell transplantations (8.61%) performed between 2.09 to 13.97 years of age (median 6.77 years). Ten patients remained on hydroxyurea but on a different scheme and 1 attained a marrow rejection. One patient died of graft-versus-host disease. Patients lost in follow-up summed up 128: 23 for emigrating to other countries, 65 for continuing the monitor of their diseases in other centers or in adults units and 31 for unknown reasons (10%).

Summary/Conclusions: Early diagnosis like universal neonatal screening allows an effective health education, and antibiotic and osteopenia prophylaxis in children with SCD. Vitamin D and general and specific vaccination can be started.

PB2144
COMPLEMENT ACTIVATION IN PATIENTS WITH SICKLE CELL DISEASE IS ASSOCIATED WITH HIGHER HBS LEVELS
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Background: Older studies have suggested activation of the alternative pathway (APC) in sickle cell disease (SCD). Despite the renewed interest in SCD therapeutics, little is known about APC activation in the clinical setting of SCD, possibly due to the complexity of complement diagnostics.

Aims: We investigated firstly, whether complement activation can be detected in the sera of asymptomatic SCD patients using a simple functional assay, secondly whether it is associated with clinical parameters and thirdly whether it can be blocked in vitro by the complement inhibitor eculizumab.

Methods: Consecutive asymptomatic SCD patients were enrolled prospectively from November 2016 to January 2017. Patient history, clinical and laboratory data were recorded. Complement activation was detected in patient sera using the modified Ham test, a cell proliferation assay based on the susceptibility of PNH (paroxysmal nocturnal hemoglobinuria)-like cell line to complement activated serum. Normal human serum (NHS) was used as a negative control and lipopolysaccharides (LPS)-incubated normal serum as a positive control. All samples were tested in triplicates and twice. Eculizumab containing serum (ECU) was added to patient serum to evaluate the effect of a complement block in vitro. The results overall showed significant differences between patients and controls (p = 0.001 and 0.001 respectively) and in verbal tasks that involved Executive Functions (inhibition and planning). APC activation during a painful crisis and the role of hydroxyurea need to be further investigated in larger series validating the role of different functional assays. Effective inhibition of complement activation in vitro is promising for future studies in selected patients.

PB2145
THE ROLE OF EXECUTIVE DYSFUNCTIONS IN THE VERBAL LANGUAGE DEFICITS OF CHILDREN WITH SICKLE CELL DISEASE
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Background: Children with Sickle Cell Disease (SCD) frequently present impairment of general and specific neurocognitive functions, even in the absence of clear neurological damage at brain neuroimaging. Verbal language deficits are also common, but the etiology of poor performance in the verbal domain is still not clear. The ability to speak and communicate verbally relies on a complex interaction of cognitive and linguistic functions as well as on environmental factors, like bilingualism or second language (L2) learning. The majority of children with SCD in Italy are immigrants whose first language is not Italian. These children thus perform poorly in tests assessing the verbal domain with adverse impact on school performances.

Aims: To evaluate if verbal language deficits in bilingual children with SCD are mainly due to linguistic and environmental issues or to impairment of executive functions.

Methods: In this study a cohort of bilingual children with SCD and social-demographically matched healthy controls recruited from elementary schools of the same school, performed an extensive battery of tests to assess naming skills, phonological and semantic fluency, attention and Executive Functions (inhibition and planning skills) and visuo-spatial skills (Boston naming test, phonological and semantic fluency tests, Flanker task, Eithorn test, PMa spatial relations sub-scale). All tests were administered in Italian. A composite index considering parental education and employment was used to match socially, demographically and educationally children with SCD. Children's Performance (in Z scores) were performed to test differences between the two groups in verbal language, attention and executive functions. Hierarchical regressions explored the contribution of linguistic knowledge and executive functions (i.e. inhibition) to the verbal language deficit of children with SCD.

Results: Thirty three children with HbSS SCD aged 6 to 12 years (mean age= 9.03) and 35 controls (mean age= 9.14) were enrolled. Patients and controls were matched for gender (F 53 vs 61%), ethnicity (African 30 vs 29%), % of children born in Italy (81 vs 80%), number of years lived in Italy (8.09 vs 8.31) and Socio-Demographic Index (5.15 vs 4.59). Children's Performance (in Z scores) at Visuo-Spatial, Boston Naming, Phonological Fluency and Semantic Fluency Tests are shown in Figure 1. The results overall showed significant differences between patients and controls in inhibition and planning (p = 0.001 and 0.001 respectively) and in verbal tasks that involved Executive Functions more (i.e. phonological fluency) (p=0.004). The poorer verbal performance of children with SCD was not related to visible lesions to Broca’s area. In fact only 9 patients presented Silent Infaracts that were all in the white matter, in watershed areas. Regression analyses showed that in children with SCD inhibition skills explained unique variance in phonological fluency, suggesting that poor executive control...
was a factor of the lower performance in this task. Figure 1. Children’s Performance (in Z scores) at Visuo-Spatial, Boston Naming, Phonological Fluency and Semantic Fluency Tests. P-values: Visuo-spatial intelligence: not significant(ns); Boston naming: ns; Phonol-Fluency: 0.004; Semantic fluency: ns.

at home to manage the onset of an acute crisis and the top 5 home strategies reported were: prescription pain medication (15%), sleep/rest (15%), apply heat using heating pad/blanket/bath/shower (13%), increase fluid intake (12%), and finally avoid stress (9%). Further it is clear, that people living with SCD are motivated to try a new therapy that could provide “significant relief” and “prevent symptoms from happening” due to their SCD.

Summary/Conclusions: The survey collected feedback about topics for which the patient is the best source of information. It is obvious that people with SCD are willing to self-medicate by subcutaneous injections and that there is a need for new tools and medications. With support from the answers from the survey, specific aspects will be considered while designing a first clinical study for subcutaneous sevuparin/DF02 administration to treat early symptoms of painful crisis in an at-home setting.

PB2147

LONG-TERM USE OF HYDROXYUREA IN CHILDREN AND ADOLESCENTS WITH SICKLE/βETA THALASSEMIA

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Background: Hydroxyurea (HU) has lately been used in the treatment of patients with severe sickle cell disease (SCD). Despite documented benefits on laboratory and clinical parameters in SCD patients, there are few reports about drug’s long-term safety and efficacy in pediatric patients with SCD – even more so in the rare patient subgroup of sickle/βeta thalassemia.

Aims: A prospective, long term evaluation of HU efficacy and safety in children and adolescents with sickle/βeta thalassemia (S/b thal).

Methods: Ten patients with S/b thal aged 3.5-18 years were followed for a 6 year period (Jan 2011- Dec 2016). HU was given at a daily dose that ranged from 10 to 20 mg/kg, with a mean of 14.1 mg/kg. Laboratory follow-up consisted of WBC, Hb, Ht, RBC, reticulocyte count and PLT count measured every 2 weeks until dose escalation to a stable dose, biochemistry assessed every 2 months and Hb F measured every 2-3 months. Patients were clinically evaluated prior to HU treatment and every 12 weeks during the study period. Evaluated data on clinical course included frequency of vaso-occlusive crises, hospitalizations and transfusions, as well as presence of severe clinical events. Hematologic toxicity of hydroxyurea was defined as a more than 20% decline from baseline in Hb, as an absolute neutrophil count of less than 1,000/μl and/or a PLT count of less than 80,000/μl. Moreover, presence of alopecia, rash, skin hyperpigmentation or headache was reported as drug-related toxicity.

Results: A significant reduction in vaso-occlusive crises as compared to prior to HU treatment was noted (median: 1 episode per year before HU, range: 0-2.5 vs median: 0.24 episodes per study year after HU, range: 0-1.33, p=0.011). A significant reduction in hospitalizations was also reported (median: 1 per year before HU, range: 0-2.5 vs median: 0.16 per study year after HU, 0-0.83, p=0.005). None of the patients presented with severe clinical events such as acute chest syndrome, avascular bone necrosis, stroke or splenic sequestration during the study period. With regards to hematological parameters, a significant increase in HbF (10.2±6.5% vs 16.6±7.1% p=0.02), MCV (66.1±3.9fl vs 79.3±8.4fl, p=0.001) and MCH (20.9±1.2pg vs 25.3±2.9pg, p=0.001), as well as a decrease in reticulocyte count (7.7±3.3% vs 5±0.9%, p=0.039), WBC count (9.566±3.674/μl vs 7.466±4.460/μl, p=0.009) and PLT count (333,778/μl±170,227 vs 272,111±160,304/μl, p=0.007) was noted. Concerning adverse events, one patient presented with mild transaminasemia, one with elevation of serum creatinine levels and one with pancytopenia. Due to persistent pancytopenia HU treatment was discontinued in the last mentioned patient, but was restarted a year later due to frequent vaso-occlusive events - despite the patient being put on transfusions after initial HU discontinuation. Besides the pancytopenia episode, the rest of the mentioned toxicities were significant and dose-dependent.

Summary/Conclusions: The study indicates that HU has an overall safe profile and results in a marked improvement of clinical course in pediatric S/b thal patients.
IN VITRO AND IN VIVO EVIDENCES OF SICKLING REVERSAL INDUCED BY REHYDRATION WITH HIGH K+-ISOTONIC SOLUTION

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Background: Erythrocyte sickling and adhesion are favoured by cellular dehydration, which increases the rate of hemoglobin polymerization and cell sickling. Potentially, high potassium co-transport and calcium-activated potassium channel (Gardos channel) mediate erythrocyte dehydration in sickle cell disease and β-thalassemia. We investigated the in-vitro and in-vivo effects of various concentration of K⁺ ions in physiological solutions (PSS) as well as in cocos nucifera water (CNw) which is known for its natural high potassium content and isotonicity.

Aims: Study was aimed at ascertaining the efficacy of isotonic potassium solutions in rehydrating sickle cell and possibly reversing the sickling phenomenon in vivo and in vitro situations.

Methods: 1. Erythrocytes from twenty sickle cell anemia (SCA) as well as 46 healthy subjects were studied. One part was treated with sodium metabisulphite (Na2S2O7) solution to induce maximum sickling as controls while the other was subjected to different high concentrations of K⁺ in PSS as well as Cocos nucifera water (40mM, 80mM and CNw - 65mMOL) respectively. The procedure was repeated for the normal HB AA subjects. Also, both groups of subjects were given 10ml/kg body weight of coconut water to drink as a single dose for the in-vivo experiment. Blood samples were collected longitudinally before and after the oral ingestion, at 1hr and at 24hrs for analysis of red cell indices as well as stained blood films used to ascertain the percentage sickled erythrocytes count before and after the treatment in both cases.

Results: Maximum percentage counts of sickled cells after the addition of Na2S2O7 (45%) were observed which decreased significantly (P<0.05, respectively) to about 2% with Cocos nucifera and 10% with 80mM K⁺PSS. The count in 40mM K⁺PSS was not statistically significant. In both HB AA and SS subjects, MCHC and MCV remained relatively stable whereas the pre-injection sample count (P>0.05, respectively) while MCHC increased significantly in both groups as early as 1hr and sustained till the 24th hour. MCHC was equally raised in the in-vitro samples (P<0.05, respectively). The morphology of red cells also indicated a lesser count of sickled red cells after the oral ingestion.

Summary/Conclusions: Cocos nucifera water and other high potassium ion solutions can activate the rehydration of sickle erythrocytes by probably de-activating the Gardos channel to increase the mean corpuscular haemoglobin concentration (MCHC) and thereby restoring the normal red cell shape. We suggest a probable pharmacological value of the cocos nucifera water as well as other formulated high potassium but isotonic fluids in SCA management.

PB2149

VITAMIN D IN SPANISH CHILDREN WITH HEMOGLOBINOPATHIES.

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Background: Although vitamin D deficiency has been documented as a frequent problem in studies of children, there are limited data on the prevalence of this nutritional deficiency among children who suffer from sickle cell disease (SCD) or thalassemia. Vitamin D homeostasis is important to prevent osteopenia. Furthermore vitamin D deficiency has been associated with increased risk of common cancers, autoimmune diseases, hypertension, and infectious diseases. Vitamin D deficiency is now recognized as a pandemic. The major cause of vitamin D deficiency is the lack of sun. Although Spain has a high rate of sunny hours, we have found low levels of vitamin D in our patients with SCD or thalassemia.

Aims: The purpose of this work is to assess the status of vitamin D in children with SCD and thalassemia in our setting.

Methods: We have recruited children diagnosed with SCD and thalassemia between 1998 and 2016 and we have reviewed their vitamin D levels. We have chosen the first vitamin value we obtained and the last one till today. Vitamin D was measured by quantitative determination of 25(OH) D. Deficit of vitamin D was defined by <30 ng/ml. The study enrolled 114 children. Most of them, with SCD diagnosis (94%). The type of anemia was Hb SS (94 patients), Hb SC (8 patients), Hb Sβ0 (3 patients) and HbSβ+ (2 patients). The remaining 6% were diagnosed with Thalassemia Major. Mostly of the children were African or Central-South American. In our centre, vitamin D prophylaxis is made since the first year of life.

Results: 60% of the children had vitamin D deficiency. We have divided children into 4 groups depending on the age. When considering vitamin D first determination: mean vitamin D levels in children below 2 years old were 39.5±13.3 ng/dl, 2 to 5 years old 35.5±13.3 ng/dl, and five years old had a mean serum vitamin D of 35.5±14.6 ng/dl. Children aged between five and ten had 26.1±13.5 ng/dl of mean 25(OH)D. Finally in the group older than 10, we observed mean of 7.4±14 ng/dl. When having these low levels of vitamin D, we strongly recommend to start treatment with Cholecalciferol 25000U/month. Regarding second levels of vitamin D, we have divided patients into those who presumably have the treatment for children who do not. We present the results in the following Table 1.

Table 1.

<table>
<thead>
<tr>
<th>Vitamin D level</th>
<th>Number of patients</th>
<th>Mean serum vitamin D (ng/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30 ng/dl</td>
<td>69</td>
<td>32.9±13.7</td>
</tr>
<tr>
<td>30-50 ng/dl</td>
<td>43</td>
<td>35.1±13.3</td>
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<td>13</td>
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<tr>
<td>&gt;70 ng/dl</td>
<td>9</td>
<td>53.9±13.5</td>
</tr>
<tr>
<td>No treatment</td>
<td>4</td>
<td>26.1±13.5</td>
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</tbody>
</table>

Summary/Conclusions: The study found a high prevalence of vitamin D deficiency in children older than five years old (in the first determination) with SCD on beta-thalassemia Major and significant decrease of levels in those not having vitamin D therapy. It is not well known the physiopathology of this factor deficiency, although it is supposed to be multifactorial. However we confirm that living in a sunny geographical situation with a healthy diet is not enough to maintain adequate 25(OH)D levels. Although the correlation of serum vitamin D levels of vitamin with oral treatment, vitamin D levels increase when having correct doses. We have also checked that older children have lower levels of vitamin D than younger boys. This could be explained by the fact that pre-teenagers spend lot of time at home instead of going out. If prophylaxis is made not only the vitamin levels will increase but bone growth also.

PB2150

KNOWLEDGE OF SICKLE-CELL DISEASE IN HAUTE-NORMANDIE, SOCIO-DEMOGRAPHIC CONTEXT AND HEALTH CHARACTERISTICS: INTEREST OF THE IMPLEMENTATION OF A PATIENT EDUCATION IN SICKLE CELL DISEASE

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Background: Sickle cell anemia (SCA) is a genetic disease causing a severe disease manifesting by painful crisis but which can also be marked by organ complications. Mortality is still happening at a young age. Many of these complications may be better taken care of if treated early. The best way to manage this disease is probably through Patient Education (PE). Sickle cell education and patient counseling in SCA has been a subject of research, organized in France by association such as ROFSED, but PE in adult patients has been little studied. The main objective of this work was to evaluate SCA patients followed in Haute-Normandie, from a sociodemographic, health and socio-demographic perspective in order to establish a PE program. The secondary objective was to give them the opportunity to express their expectations of such a program.

Methods: We did an observational multicenter study. A self-questionnaire of 39 items was sent to all patients suffering from SCA followed in Haute-Normandie.

Results: Fifty patients (male / female ratio 0.92) out of 123 (40.6%) responded, mean age 33±10.5 years (SS genotypes [66%], SC[25%], S-beta-thalassemia [9%]), 56% of them were born outside of Metropolitan France, 36% came from French speaking African countries. Age range was age 18±10.9 years. Despite the fact that their education has been disrupted by the disease for the majority (69.4%), the level of education was satisfactory (31.3%) or high (6.8%) which is all the more surprising as 10% had a primary / middle school level and 4% were illiterate. 68% of the patients had a job or were students. 48% of patients reported to practice physical activity at least once weekly. Tobacco was consumed on a daily basis by 14%, alcohol 2% and 4% for cannabis. Self-assessment of health status was 6.9 / 10, self-assessment of morale of 7.9 / 10 and impact of the disease on daily life was estimated at 5.4 / 10. The mean age at which specialized follow-up was started was 11±9 years. 88% of the subjects stated that they understood everything the doctor said during consultation. Missed appointments were reported by 26% which was justified by forgetfulness, lack of will or physical incapacity. Regarding sources of information regarding SCA, patients declared asking their specialist first and then looking on the internet. 68% of patients had a first-degree relative suffering from the same disease, 71% were able to talk about the disease with their family. While the triggers of crises and the management of crises were well-identified by patients (average scores of 13.8 and 12/20), “standards” were not met with chronic complications, prenatal diagnosis, and long term treatment (mean scores respectively of 7.4; 4.2 and 2.2 / 20). Average score on the whole questionnaire was 9/20. Most patients showed interested in PE (52.1%) vs 31.3% that claimed were not interested. 17.7% did not decide.

Summary/Conclusions: A majority of SCA adults followed in Haute-Normandie are first-generation migrants. Even if the disease has heavy impact on everyday life and school access, their education level appeared correct. PE sessions will not make the directives explicit to the patients, but they could be a form of treatment. The majority of adults with SCA are motivated by PE, we will have to adapt to a heterogeneous population in terms of educational level, ethnic origin and knowledge of the disease.
**PB2151**

**DELAYED HAEMOLYTIC TRANSFUSION REACTIONS: A MASQUERADE OF SICKLE CELL COMPLICATIONS**

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**Background:** Patients with sickle cell disease (SCD) may require repeated red blood cells (RBCs) transfusion, putting them at risk from minor blood group alloimmunization and the development of delayed haemolytic transfusion reactions. Aims: To report a prevalence of recognized DHTR syndrome in patients with SCD. Methods: We reviewed the cases of (DHTR) in SCD patients in a 5-year period (2010-2016). A total of 10 patients had a clinical picture compatible with DHTR and underwent treatment with high dose steroids, intravenous immunoglobulins (IV Ig) or ephrithropoetin. Any patient received Rituximab.

**Results:** The most common indications for transfusion were anemia due to vasococclusive sickle cell crisis or preoperative anaemia optimization. The cohort received partial exchange transfusion and phenotypically matched RBCs. Before transfusion the median of Hb level was 69 g/L (baseline range 80g/L) and the nadir at haemolysis episode was 38 g/L. HI was 21.9%, WBC was 17.3 × 10^9 cells/L and mean LDH 1290 IU/L. The median time to develop DHTR was seven days after the transfusion and approximately 6 days after the surgical interventions (range: 4–12 days) and all cases presented with symptoms of anaemia, jaundice, tiredness and tachycardia. The median age was 29 years with female predominance (6:4). Blood cultures were negative in 80% of patients and only positive in 2 cases. 30% of patients tested positive for viral infection on PCR. Mortality rate in our series was low (zero). Pain episodes and other complications associated with DHTR was treated as required and four cases were successfully monitored in HDU. One patient required noninvasive ventilations and inotropic support. Two patients received RBC transfusion for 2 units of packed RBC. Possibly as their presence tachyphylaxis mimics an acute vasococclusive crisis. In all cases haemoglobin stabilized and improved, symptoms resolved and patients were discharged on small course of oral antibiotics (median admission 6 days).

**Summary/Conclusions:** The symptoms of DHTR can easily be mistaken for other SCD complications, including infection and vaso-occlusive crisis. The diagnosis of DHTR is based on clinical suspicion, when there is a rapid Hb drop after a recent RBC transfusion with clinical signs of haemolysis. To support the diagnosis, laboratory tests (serial FBCs, haemolysis screen, DAT, measurement of Hb S levels) and exclusion of other aetiologies are useful. Whenever a DHTR is suspected, further RBC transfusion should be withheld until absolute necessary, as it may precipitate acceleration of the hemolytic reaction. Patients in whom the diagnosis of DHTR is missed may receive repeat transfusions, which may contribute to the complications associated with SCD. The use of more extensive phenotypic matching of blood and minimizing RBC transfusion help to prevent DHTR. The present series emphasizes the importance of early recognition of symptoms and signs in correlation with a recent history of RBC transfusions, as DHTR can be a potentially life-threatening complication.

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**PB2152**

**HBS MONITORING ON TOSOH G8 IN VARIANT HBA1C MODE IN CASE OF URGENT RCE**

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1Department of Laboratory Medicine, University Hospitals Leuven, 2Department of Cardiovascular Sciences, 3Department of Microbiology and Immunology, KU Leuven, Leuven, Belgium

**Background:** Pre- and post-transfusion HbS levels are used to document the efficacy of red blood cell exchange (RCE) in patients with sickle cell disease (SCD). In case of urgent RCE a 24/7 STAT analysis, with the ability to identify and quantify hemoglobin (Hb) S, is warranted. **Aims:** We evaluated the use of Tosoh Automated Glycohemoglobin Analyzer HLC-723G8 (Tosoh Europe, Amsterdam, The Netherlands) for this purpose, using the variant HbA1c mode. Results were compared to our routine CZE Minicap Flex Piercing (Sebia, Lisses, France) for this purpose, using the variant HbA1c mode. Results were compared to our routine CZE Minicap Flex Piercing (Sebia, Lisses, France).

**Methods:** We reviewed the cases of (DHTR) in SCD patients in a 5-year period (2010-2016). A total of 10 patients had a clinical picture compatible with DHTR and underwent treatment with high dose steroids, intravenous immunoglobulins (IV Ig) or ephrithropoetin. Any patient received Rituximab.

**Results:** The most common indications for transfusion were anemia due to vasococclusive sickle cell crisis or preoperative anaemia optimization. The cohort received partial exchange transfusion and phenotypically matched RBCs. Before transfusion the median of Hb level was 69 g/L (baseline range 80g/L) and the nadir at haemolysis episode was 38 g/L. HI was 21.9%, WBC was 17.3 × 10^9 cells/L and mean LDH 1290 IU/L. The median time to develop DHTR was seven days after the transfusion and approximately 6 days after the surgical interventions (range: 4–12 days) and all cases presented with symptoms of anaemia, jaundice, tiredness and tachycardia. The median age was 29 years with female predominance (6:4). Blood cultures were negative in 80% of patients and only positive in 2 cases. 30% of patients tested positive for viral infection on PCR. Mortality rate in our series was low (zero). Pain episodes and other complications associated with DHTR was treated as required and four cases were successfully monitored in HDU. One patient required noninvasive ventilations and inotropic support. Two patients received RBC transfusion for 2 units of packed RBC. Possibly as their presence tachyphylaxis mimics an acute vasococclusive crisis. In all cases haemoglobin stabilized and improved, symptoms resolved and patients were discharged on small course of oral antibiotics (median admission 6 days).

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SICKLE CELL PAIN IN CHILDREN: TARGETS FOR ADMINISTRATION OF ADEQUATE INITIAL ANALGESIA
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Background: Acute pain is a hallmark presentation in sickle cell disease (SCD) and frequently requires attendance to the emergency department (ED).

Aims: Here we report our findings following a complete retrospective audit cycle, documenting the timing of analgesia administration and post-treatment pain review as per National Institute of Clinical Excellence and College of Emergency Medicine guidelines, in children with SCD presenting to a single inner city London ED over a 14 month period.

Methods: In 2014, we evaluated 48 patient records of children presenting to the ED, with regard to mild, moderate and severe pain scores, time of analgesia administration and pain review. Completing the audit cycle, 97 records were re-audited in 2015. A total of 145 admission records were evaluated.

Results: In 2014 the ED met CEM criteria for the timeliness of analgesia administration in 100% of severe and 95% of the moderate pain category; however fell 33% short of NICE standards. Pain review was poorly performed, identifying an area for improvement. Proportions meeting the aforementioned criteria fell significantly in 2015, except review of moderate pain, which increased by 25%.

Summary/Conclusions: We conclude CEM guidelines promote timely administration of analgesia in patients with severe pain; however mild pain may be overlooked. NICE avoids this discrimination. Thus we recommend combining the mild and moderate pain categories to acknowledge the fluctuating nature of sickle pain and its tendency to rapidly escalate. In addition, we reiterate the need for regular pain reviews. This is important in ensuring analgesia is closely titrated to pain level.

PB2156
DIAGNOSTIC CHALLENGES IN A POPULATION WITH INCREASED IMMIGRATION: HEMOGLOBINOPATHIES IN THE NEW CENTURY
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Background: The diagnosis of hemoglobinopathies (Hbpts) has changed in recent years due to immigration, with an increase in structural Hbpts. In our area there is a predominance of β-thalassemia minor. Structural Hbpts are the main diagnosis in immigrants. The incidence is still small, although increasing in the last 3 years, so a neonatal screening program is being implemented. Both HPLC and CE are simple, fast and efficient methods in the diagnosis of Hbpts. In our area, anemia are mainly due to β-thalassemia minor. Structural Hbpts are the main diagnosis in immigrants. The incidence is still small, although increasing in the last 3 years, so a neonatal screening program is being implemented. Both HPLC and CE are simple, fast and efficient methods in the diagnosis of Hbpts.

Methods: We analyzed 1202 patients, 49% were males and the median age in the last 10 years.

Results: In 2014 the diagnosis of Hbpts and thalassemias in our region was a predominance of β-thalassemia minor. Structural Hbpts are the main diagnosis in immigrants. The incidence is still small, although increasing in the last 3 years, so a neonatal screening program is being implemented. Both HPLC and CE are simple, fast and efficient methods in the diagnosis of Hbpts.

Summary/Conclusions: In our area there is a predominance of β-thalassemia minor. Structural Hbpts are the main diagnosis in immigrants. The incidence is still small, although increasing in the last 3 years, so a neonatal screening program is being implemented. Both HPLC and CE are simple, fast and efficient methods in the diagnosis of Hbpts.
Summary/Conclusions: L-arginine supplement should be made available in the paediatric emergency unit, clinic and pharmacy department in high risk communities to obviate the negative effects during vaso-occlusive crisis and potentially reduce the length of stay in the hospital. L-arginine, nitric oxide, total antioxidant capacity, malondaldehyde and glutathione levels should be routinely monitored in sickle cell disease patients particularly those presenting with vaso-occlusive crisis.

Stem cell transplantation - Clinical

PB2157

THE EFFECT OF BODY MASS INDEX ON OUTCOME AFTER UMBILICAL CORD BLOOD TRANSPLANTATION IN PEDIATRIC PATIENTS WITH ACUTE LEUKEMIA ON BEHALF OF EUROCORD, PDPW

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1EUROCORD, Hopital Saint Louis, Paris, France; 2MONACORD, Centre Scientifique de Monaco, Monaco, Monaco; 3Hematology and Immunology, Hospital Saint Antoine, 4Pediatric Hematology Department, Hospital Robert Debré, Paris, 5Pediatric Hematology Department, Hospital De la Timone, Marseille, France; 6Department of Pediatric Hematology-Oncology, IRCCSS Ospedale Bambino Gesù, Rome, Italy; 7Pediatric Onco-Hematology Department, Hôpital des Enfants, Bordeaux, France; 8Department of Cellular Biotechnologies and Hematology, Sapienza University, Rome, Italy; 9UAM allo-CORD CHRU, Hôpital HURIEZ, Lille, France, 10Hematology-Oncology Division, C.H.U. Saint-Justine, University of Montreal, Montreal, Canada; 11Hematology Department, Hospital Infantil Universitario Nino Jesus, Madrid, Spain; 12Institute of Hematology and Oncology Paediatrics, Hospices Civils de Lyon, Lyon, France; 13Hospital Sírio Libanês, Sao Paulo, Brazil; 14Division of Stem Cell Transplantation and Immunology, Hospital for Children and Adolescents of Frankfurt, Frankfurt, Germany

Background: Body mass index (BMI) may influence outcome after allogeneic transplantation. Previous studies have demonstrated that being obese or underweight may have a detrimental effect on survival rates after chemotherapy induction in children with acute leukemia. However, the impact of BMI of transplanted patients on survival is still not clear, with conflicting results being reported on this issue.

Aims: To analyze the effect of BMI on UCBT outcomes in children with acute leukemia

Methods: We retrospectively analyzed 517 patients aged from 2 to 20 years with acute leukemia who underwent umbilical cord blood transplantation (UCBT) from 1990 to 2015. Patients were classified according to BMI as normal (5th-85th percentile), underweight (<5th percentile), overweight (85th-95th percentile) and obese (>95th percentile) by using growth charts for age and gender.

Results: Sixty-one percent (n=314) of patients were in the normal category, 12% (n=63) were underweight, 15% (n=80) overweight and 12% (n=80) obese. All patients received single-unit UCBT after a myeloablative conditioning regimen. Diagnosis was acute lymphoid leukemia in 70% (n=363) and acute myeloid leukemia in 30% (n=154). Median age at UCBT was 7.4 years (range 2-19.6). Cytomegalovirus (CMV) serology was positive in 45% patients; 60% of patients were male. Most patients (92%) were in complete remission at UCBT. Median follow-up was 52 months (range 2-201). Total body irradiation (>6 Gy) was used in 58% of cases; antithymocyte globulin (ATG) in 68% of cases. Median infused total nucleated cell (TNC) dose was 4.2x10^7/Kg (0.3-17.8); 56% of patients received a graft with 0-1 HLA mismatch donor. Four-year overall survival (OS), leukemia-free survival (LFS) and non-relapse mortality (NRM) was 22.8% (19.2-26.7%). In univariate analysis, no statistically significant difference in OS, LFS, GRFS, neutrophil engraftment, NRM and chronic GVHD between the 4 groups identified according to BMI was identified. Conversely, acute GVHD was 44.3% (33.3-58.8%) for underweight, 36% (31-41.8%) for normal, 26.2% (18-18.3%) for overweight and 23.3% (14.7-37.1%) for obese (p=0.03). Among patients underweight who experienced acute GVHD (n=27), 37.5% had grade III-IV acute GVHD with gut involvement. In multivariate analysis, infused TNC dose>4.2x10^7/Kg was associated with higher neutrophil engraftment (HR=1.46, CI 95% 1.19-2.78, p<0.001), positive CMV serology (HR=1.5, CI 95% 1.04-2.28, p=0.03) and female gender (HR=1.5, CI 95% 1.03-2.23, p=0.03) were associated with higher NRM. ATG use (HR=1.6, CI 95% 1.05-2.31, p=0.03) was associated with higher relapse incidence. Moreover, ATG use and a positive CMV serology were associated with worse OS (HR=1.6, CI 95% 1.15-2.17, p=0.04) and HR=1.3, CI 95% 1.01-1.69, p=0.001, respectively) and LFS (HR=1.6, CI 95% 1.17-2.16, p=0.001 and HR=1.3, CI 95% 1.04-1.72, p=0.02, respectively). Infused TNC >4.2x10^7/Kg (HR=1.5, CI 95% 1.07-2.14, p<0.02), lack of ATG in the conditioning (HR=2.72, CI 95% 1.6-3.1, p<0.001) and BMI <5th percentile (HR=1.8, CI 95% 1.19-2.78, p=0.001) were associated with higher incidence of acute grade II-IV GVHD.

Summary/Conclusions: In conclusion, we did not find association of obesity with transplant outcomes in this study population. However a BMI <5th percentile at UCBT was found to be associated with higher risk of acute GVHD, highlighting the importance of nutritional status before UCBT.
PB2158

PROSPECTIVE PHASE STUDY OF REDUCED TOXICITY CONDITIONING CONSISTED OF HIGH DOSE CYTARABINE, FLUDARABINE, CYCLOPHOSPHAMIDE +/- TOTAL BODY IRRADIATION FOLLOWED BY ALLOGENEIC STEM CELL TRANSPLANTATION


1Hematology, Gifu University Graduate School of Medicine, 2Hematology, Gifu Red Cross Hospital, 3Internal Medicine, Gihoku Kosei Hospital, Gifu, Japan

Background: Allogenic hematopoietic stem cell transplantation (allo-SCT) using reduced intensity conditioning (RIC) has been widely applied to elderly or frail patients who are not eligible for conventional conditioning regimen. However, benefit provided by reduced toxicity has been often offset by increased incidence of relapse. So far, the optimal conditioning for those patients has not been established.

Aims: Here, we investigate whether addition of high dose cytarabine ( AraC) to RIC regimen consisting of fludarabine (Flu) and cyclophosphamide (Cy) +/- total body irradiation (TBI) can be available for elderly or frail recipients, phase II study has been designed.

Methods: This study was conducted from April 2011 to December 2015. The protocol was approved by each institutional review board (Trial identifier: UMIN000007281). Patients aged from 55 to 70, or patients who have some organ damage or a history of SCT aged from 20 to 54 with hematologic malignancies were enrolled after obtaining written informed consent. Bone marrow (BM), peripheral blood (PB), or cord blood (CB) was used as stem cell sources. Pretransplant conditioning regimen consisted of 30 mg/m2 of Flu for 5 days (total 150 mg/m²), 4 g/m2 of AraC for 2-4 days (divided by 2 daily, total 8-16 g/m²) and 50mg/kg of Cy for a day. Four gray of TBI was used for all CB transplant recipients, whereas 2 gray of TBI was used in other stem cell sources. Donor cell engraftment and 60 day-survival were assessed as a primary end point to evaluate feasibility of this protocol.

Results: Thirty nine patients including 7 recipients with a history of SCT were enrolled. Median age was 61 (28-69), 21 were male, and 18 were female. Nineteen were acute myelogenous leukemia, 11 myelodysplastic syndrome, 6 malignant lymphoma and 3 acute lymphoblastic leukemia. Donors were 4 matched related donor as a source for aloSCT in Hodgkin's disease. Despite the promising results, we have no statistical power to conclude the efficacy of this protocol.

Conclusion: We performed a retrospective analysis of patients with NHL that received an ASCT between October 1992 and December 2012. The late complications were defined as those to other previous comorbidity or to aging. Statistical analysis was performed using the IBM SPSS Statistics version 21.0. Cumulative incidences were estimated using EZR version 1.27 (Saitama Medical Center, Jichi Medical University, Omiya, Japan), a graphical user interface for R (version 3.1.1).

Results: A total of 105 allografted patients were analyzed. Patient’s characteristics are in Table 1. The median follow up since ASCT was 73 months (0 – 274 months). Thirty-one percent (n=33) of patients were conditioned with CFM-TBI. The overall 5-years survival (OS) was 68.3% (58-77% - CI 95%) and the 5-year disease free survival (DFS) was 52% (42 61% - CI 95%). There were no differences regarding OS and DFS between the two conditioning regimens.

The 5-years cumulative incidence (CI) of relapse was 0.48 (0.37-0.57. CI 95%). We detected 10 secondary neoplasm (myelodysplasia n=1, skin carcinoma n=2, lung carcinoma n=3, oropharangeal carcinoma n=1, intestinal adenocarcinoma n=1, renal neoplasia n=1, bladder neoplasia n=1). The median time for the neoplastic event was 10.5 years (0-18.5 years). The CI of secondary neoplasia (2nd neoplasia) at 10 years was 10% (1-20%, CI 95%) and at last point of follow up (18.5 years) was 40% (13%/63%, CI 95%). There were no differences in the CI of 2nd neoplasias between BEAM and CFM-TBI. Non-neoplastic complications were present in 10% of patients (n=11). Three cases were infections grade 3-4 related to ASCT. Six cases had cardiac complications (5 acute coronary syndrome, 1 myocardiopathy) and 2 had pulmonary toxicity. The CI of non-2nd neoplastic complications at 10 year was 10% (1-25%, CI 95%).No differences were detected between the two conditioning regimens regarding non-neoplastic complications. (see Figure 1).

Table 1. Patient’s characteristics.

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<td>66-70 year</td>
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Summary/Conclusions: We performed a retrospective analysis of patients with NHL that received an ASCT between October 1992 and December 2012. The late complications were defined as those to other previous comorbidity or to aging. Statistical analysis was performed using the IBM SPSS Statistics version 21.0. Cumulative incidences were estimated using EZR version 1.27 (Saitama Medical Center, Jichi Medical University, Omiya, Japan), a graphical user interface for R (version 3.1.1).

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Figure 1.

Summary/Conclusions: Autologous stem cell transplantation offers long disease free survival for half of the patients with a high risk non-Hodgkin lymphoma. In our series, patients conditioned with BEAM or CFM-TBI had a comparable incidence of neoplastic and non-neoplastic events

PB2159

LATE COMPLICATIONS OF CONDITIONING REGIMENS (CYCLOPHOSPHAMIDE - TOTAL BODY IRRADIATION vs BEAM) FOR AUTOLOGOUS STEM CELL TRANSPLANTATION IN NON-HODGKIN LYMPHOMA

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Background: Autologous stem cell transplantation (ASCT) is a frequently used procedure for the treatment of patients with relapsed non-Hodgkin lymphoma (NHL). While chemotherapy-based regimens are now commonly administered, total body irradiation (TBI) was largely used in the past. The current conditioning regimen in our center is BEAM (a combination of carmustine (BCNU), etoposide, cytarabine and melphalan) although we also have a large experience with cyclophosphamide (CFM)-total body irradiation (TBI) since this was the usual conditioning until year 2000.

Aims: To analyze the cumulative incidence of secondary neoplastic complications (grade 3-4 infections, cardiovascular and pulmonary toxicity) after the two conditioning regimens (CFM-TBI vs BEAM) for ASCT.

Methods: We performed a retrospective analysis of patients with NHL that...
Methods: We studied 127 adult patients who underwent ASCT following LEED or MEC as the conditioning regimen against chemo-sensitive ML at four institutions in Japan between 1997 and 2015. Any type of pathobiologic diagnosis was considered. The LEED regimen consisted of 140 mg/m² BCNU (days −8 and −7), 300 mg/m² cyclophosphamide (days −4 to −3), 500 mg/m² etoposide (days −2 and −1), and 40 mg/body dexamethasone (days −1 to −5). The MCEC regimen consisted of 200 mg/m² L-PAM (day −1), 500 mg/m² etoposide (days −4 to −2), 60 mg/kg cyclophosphamide (days −3), and 40 mg/body dexamethasone and 40 mg/body dexamethasone on day −1. The patients who relapsed and in 70% (7) in the non-relapsed group. 38% of the whole group of patients, had a donor/recipient KIR alloreactivity without differences between the two groups of the study. 88% (7) of the relapses occurred before 6 months of the SCT. The mean time to relapse was 316 days (range 181-446). Between the 8 relapsed patients, 5 patients received another center with Vindesine / Dexamethasone and died by infection, another patient died by abdominal sepsis before starting any treatment. Brentuximab was administered in 63% (5) of the patients. One of them received a single Brentuximab cycle with no tolerance, and changed to RT, GPD+Donor lymphocyte infusions (DLI) and had reached complete remission after 5 DLI. The rest (4) received between 3 and 7 doses with adequate tolerance. According to the re-evaluation (PET-TC) after 3rd Brentuximab, 4 were in partial remission and one reached complete response. We associated Donor lymphocyte infusions in 6 patients. The mean of DLI received was 10; the median was 8, with a range between 22-34. One patients reached complete remission, two of them maintain a partial response. All of them presented good tolerance to DLI. We observed Graft versus host disease in four patients, 3 of them presented moderate cutaneous affection, and one of them suffered hepatic graft versus host disease stage III, with adequate evolution after treatment.

Summary/Conclusions: It is possible to treat patients who relapsed after haploidentical stem cell transplantation with Brentuximab+DLI, with a very good tolerance. We observed cutaneous graft versus host disease in most of the patients who reached completed response after DLI. Despite this findings, we need multicentric studies to perform standarized treatments and protocols.

PB2161
CONDITITIONING REGIMENS BEFORE AUTOLOGOUS STEM CELL TRANSPLANTATION FOR PATIENTS WITH MALIGNANT LYMPHOMA – LEED vs MCEC –
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Background: High-dose chemotherapy before ASCT has been established as an effective treatment option for high-risk patients with chemo-sensitive ML. Although the therapeutic efficacy of this strategy highly depends on the conditioning regimens before ASCT, the appropriate regimen has been controversial. Thus, we performed a multi-center retrospective study of ASCT recipients with ML to compare the safety and efficacy of the conditioning regimens LEED and MCEC, which are widely used in Japan.

Aims: The primary objective was to determine the preferable conditioning regimen before ASCT: LEED or MCEC.

Methods: This study analyzed 127 adult patients who underwent ASCT following LEED or MEC as the conditioning regimen against chemo-sensitive ML at four institutions in Japan between 1997 and 2015. Any type of pathologic diagnosis was considered. The LEED regimen consisted of 140 mg/m² L-PAM (day −1), 500 mg/m² etoposide (days −4 to −2), 60 mg/kg cyclophosphamide (days −4 to −3), and 40 mg/body dexamethasone (days −4 to −2). The MCEC regimen consisted of 200 mg/m² L-PAM (days −8 and −7), 300 mg/m² carboplatin (days −7 to −4), 500 mg/m² etoposide (days −6 to −4), and 50 mg/kg cyclophosphamide (days −3 to −2). Fisher’s exact test was used to compare binary variables. OS rates were estimated by the Kaplan-Meier method and compared using the log-rank test. Cumulative incidence statistics of relapse and non-relapse mortality were estimated considering the opposite event as competing. Fine and Gray’s method for CI of TRM and RRD was used to evaluate the risk factors on univariate analysis.

Results: Twenty-three patients were in remission on day +30, by bone marrow cytology, 3 patients were classified as resistant disease and five patients were not evaluable during CR. Because of early death. Five patients (21.7%) relapsed after a median of 6 months (range, 3 to 15). At the time of this analysis (December 2016) 14 patients were alive with a median OS of 53 months (range 20-90), while 17 patients died after a median of 4 months (range 1-27); RRD was 16% (n=5) and TRM was 39% (n=12). Non relapse causes of death were as follows: GVHD (n=3), infections complications (n=8) and EBV-related PTLD (n=1). One patient experienced a third tumor (breast cancer) thirteen years from HSCT. TRM was higher for patients transplanted from MUD (66%) as compared to REL donor (41%) (p<0.01). The overall survival was 45.2% (Figure 1) and 58% maintained a complete remission.

Summary/Conclusions: This report confirms that allogeneic HSCT is a curative approach in approximately 50% of patients with therapy related haematological neoplasms, especially for those patients who benefit from a familial donor.
IMPROVEMENT IN BIVENTRICULAR CARDIAC MECHANICS NOTED IN PATIENTS UNDERGOING MYELOABLATIVE AUTOLOGOUS-HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR AL AMYLOIDOSIS

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Background: Primary amyloidosis (AL) is characterized by extracellular deposition of insoluble protein fibrils often with multisystem organ involvement. The Mayo staging model for determining prognosis in patients with cardiac amyloidosis takes into account troponin, NT-proBNP, and serum free-light chain difference in order to stage patients prior to undergoing autologous hematopoietic stem cell transplant (Auto-HCT). Since amyloidosis often involves the kidneys, serum biomarkers that require renal clearance are less reliable in the setting of significant renal dysfunction. 2D-echo and strain imaging offer non-invasive modalities for identifying early cardiac changes independent of renal function. These changes may also precede symptom improvement as assessed by NYHA classification.

Aims: Our hypothesis is that strain imaging is a feasible biomarker for cardiac response after Auto-HCT in AL amyloidosis.

Methods: Seven patients with biopsy-proven AL amyloidosis who were treated with a Melphalan based myeloablative regimen and Auto-HCT were evaluated retrospectively. Each patient underwent 2D-echo up to 36-days prior to treatment followed by repeat 2D-echo within 14-months. Strain imaging was performed using Echolinsight®. Chart review was conducted to determine associated NYHA functional classification and Mayo staging. Statistical analysis was performed using SPSS.

Results: Of the 7 patients studied, 3 were Mayo stage I, 2 stage II, 1 stage III, and 1 stage IV. The median follow-up from transplant was 47.4 months. There was one death at 20.4 months. The mean NYHA classification at baseline was 2.3 and after transplant was 1.9. Longitudinal, radial and circumferential left ventricular strain (LV) were evaluated, but only the global longitudinal strain (GLS) showed an improvement (baseline -14.69%; follow-up -16.84%; mean absolute improvement 2.15%; p <0.05) across all four Mayo Stages. There was no difference in GLS within individual stages. In patients with stable NYHA classification after transplant, there was also a significant improvement in Right Ventricular Free-Wall Strain (RVFWS) with a mean absolute improvement of 6.2% (p <0.05). There was no significant change in left ventricular ejection fraction (LVEF) (Figure 1).

Summary/Conclusions: We demonstrate that there is a clinically meaningful improvement in cardiac mechanics one year after Auto-HCT, despite no alteration in LVEF. This metric may prove useful in assessing organ response, especially when serum biomarkers are less reliable. Changes in left ventricular GLS occur independent of pre-transplant Mayo stage, although prospective studies are needed for confirmation. We further believe that improvements in RVFWS may predict clinical improvement.

PB2164
AN ABSOLUTE NUMBER OF CD34+ CELLS IN BLOOD AS A PREDICTOR OF A SUCCESSFUL HARVEST OF HEMATOPOIETIC STEM CELLS IN DIFFERENT MOBILIZATION REGIMENS

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Background: Autologous stem cells transplantation (ASCT) has become necessary part in therapy of hematological diseases. Transfusion of at least 2×106 CD34+ HSCs per kg of patient’s weight allows achieving an adequate hemopoiesis after high-dose chemotherapy. The most optimal is to collect ≥2×106 CD34+ cells/kg with single harvest apheresis. Different mobilization regimens have been investigated in various therapies. The most optimal is to collect ≥2×106 CD34+ cells/kg with single harvest apheresis. Different mobilization regimens have been investigated in various therapies. The most optimal is to collect ≥2×106 CD34+ cells/kg with single harvest apheresis. Different mobilization regimens have been investigated in various therapies. The most optimal is to collect ≥2×106 CD34+ cells/kg with single harvest apheresis. Different mobilization regimens have been investigated in various therapies. The most optimal is to collect ≥2×106 CD34+ cells/kg with single harvest apheresis.

Methods: The study included 142 patients (pts) who undergo ASCT (80 m, 62 f; median age 53 y.o.; 81 were diagnosed with multiple myeloma, 10 - Hodgkin’s lymphoma, 51 – non-Hodgkin’s lymphomas). WBC and absolute CD34+ number in the blood before the first apheresis and the number of CD34+/kg in the apheresis product were determined for each patient. There were three different mobilization regimens: 1) chemotherapy-based mobilization: 10 mg/m2 cisplatin, 2 g/m2 cytarabine and 10 μg/kg/day G-CSF (DHAP+G-CSF); 2) chemotherapy-based mobilization: 10 mg/m2 cisplatin, 2 g/m2 cytarabine and 10 μg/kg/day G-CSF (DHAP+G-CSF); 3) chemotherapy-based mobilization: 10 mg/m2 cisplatin, 2 g/m2 cytarabine and 10 μg/kg/day G-CSF (DHAP+G-CSF). CD34+ HCS were evaluated with ISHAGE-protocol by DB FACSCanto II flow cytometer. Results are presented as mean±SEM. ROC-analysis was performed for WBC and the absolute number of CD34+ HSCs in the blood as the predictor markers for HSCs successful harvesting (≥2×106 CD34+/kg for first apheresis).

Results: WBC was higher in pts with GSF mobilization scheme compared to Cph+G-CSF and DHAP+G-CSF (28.5±3.5 vs 10.4±2.9 and 9.0±1.8×10⁶/μl, respectively, p<0.001), but the absolute number of CD34+ cells in the blood before the first apheresis and the number of CD34+/kg in the apheresis product was determined for each patient. There were three different mobilization regimens: 1) chemotherapy-based mobilization: chemotherapy plus 10 μg/kg/day G-CSF (Cph+G-CSF); 2) chemotherapy-based mobilization: chemotherapy plus 10 μg/kg/day G-CSF (DHAP+G-CSF); 3) chemotherapy-based mobilization: chemotherapy plus 10 μg/kg/day G-CSF (DHAP+G-CSF). CD34+ HCS were evaluated with ISHAGE-protocol by DB FACSCanto II flow cytometer. Results are presented as mean±SEM. ROC-analysis was performed for WBC and the absolute number of CD34+ HSCs in the blood as the predictor markers for HSCs successful harvesting (≥2×106 CD34+/kg for first apheresis).

Summary/Conclusions: We demonstrated that WBC and the number of CD34+/kg in the apheresis product is 0.952 and a threshold of successful harvesting was 20 CD34+ cells/μl in blood before apheresis with sensitivity of 96% and specificity of 81%.
PB2166
EXTRACORPOREAL PHOTOPHERESIS IN STEROID-DEPENDENT OR REFRACTORY ACUTE GRAFT-VERSUS-HOST DISEASE
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Background: Extracorporeal photopheresis (ECP) has been incorporated in the management of graft-versus-host disease (GVHD) post allogeneic haematopoietic cell transplantation (allo-HCT) in many centres. The introduction of ECP as an early second-line treatment in steroid-dependent or refractory patients with acute GVHD (aGVHD) remains under study. The rationale of its early use is based on the low incidence of complete responses to corticosteroids and the profound immunosuppression caused by traditional secondary treatments.

Aims: Based on our long-lasting experience in chronic GVHD, we aimed to prospectively assess the role of ECP in this high-risk population.

Methods: We enrolled consecutive patients with steroid-dependent or refractory grade (gr) II-IV aGVHD post alloHCT from January 2013 to August 2016. All patients with unrelated or haploidentical donors received thymoglobulin (ATG) 5mg/kg as prophylaxis. Post-transplant GVHD prophylaxis included cyclosporine – methotrexate in myeloablative and cyclosporine – mycophenolate mofetil in reduced toxicity or intensity regimens. ECP was commenced after assessment of response to 5 days of steroid treatment according to our protocol: 2 sessions/week for 1 month, 1 session/2 weeks for 3 months, evaluation of response and 1 session/month for 6 months.

Results: We studied 20 patients, aged 35 (18-65), post alloHCT with myeloablative (14), reduced toxicity (4) and intensity (4) conditioning, from sibling (13), matched (8) or one locus mismatched (8) volunteer unrelated and haploidentical (1) donor. Disease risk index was high (10), intermediate (9) and low (1). Acute GVHD was observed at day +17 (8-50) in 15 patients, late-onset at +130 (110-160) in 4 patients and induced at +38 post donor lymphocyte infusion in a relapsed AML patient. Skin, intestine and liver involvement was evident in 6 patients, skin and intestine in 10 and skin only in 4 patients. Nine patients (2 with GrIII, 7 with GrIV) steroid-refractory.  ATG was administered simultaneously with ECP initiation (autoimmune haemolytic anaemia AIHA (n=2), AIHA with acquired red Cell infection (n=1)). We assessed the reproducibility and linearity of the new device and compared numbers and viabilities of CD45+ cells and CD34+ cells determined with the ADAM IITM and flow cytometer.

Summary/Conclusions: The newly developed image-based microscopic cell counter (ADAM II™) appears to be suitable for quantification of CD34+ cell and its viability of fresh or cryopreserved PBSCs or CBs.

PB2167
RAPID RECONSTITUTION OF NK1 CELLS IS ASSOCIATED WITH THE LOWER INCIDENCE OF GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENEIC TRANSPLANTATION
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Background: The balance between immunostimulation and immunoregulation in T cell immunity is achieved by a Th1/Th2/Th3/Tr1 and CD4+CD25+ regulatory T (Treg) cell paradigm.

Aims: We investigated the production of type1 (IFN-gamma, NK1), type2 (IL-13, NK2), type3 (TGF-beta, NK3) and regulatory cytokines (IL10, Nkr) from human peripheral blood to discuss the cytokine paradigm of NK cells in human allogeneic hematopoietic stem cells transplantation (allo-HSCT).

Methods: Forty patients undergoing haploidentical (n=27) and HLA-identical sibling (n=13) allo-HSCT between August 2009 and December 2009 were enrolled in this analysis after being originally selected using a protocol exploring the association of reconstituted donor derived NK1/NK2/NK3/Nkr cells to GVHD and CMV reactivation.

Results: Expansion of NK2 and NK3 were found post allo-HSCT compared to healthy donor. The levels of Nkr reconstituted to donor’s level since day 15 post allo-HSCT, and the levels of NK1 in recipients post transplantation were consistently lower compared to donors’ levels until day 60 post allo-HSCT. Multivariate analysis showed that the higher levels of NK1 by day 15 were associated with lower overall acute GVHD (HR 0.157, 0.039-0.642, P=0.010) as well as II-IV acute GVHD (HR 0.260, 95%CI, 0.064-1.053, P=0.059). Meanwhile, the higher levels of NK1 by day 15 correlated with lower CMV reactivation (HR 0.011, 0.005-0.348, P=0.003).

Summary/Conclusions: These results indicate that rapid reconstitution of NK cells; especially NK1 cells would be helpful to prevent the development of graft-versus-host disease as well as CMV reactivation after allogeneic transplantation.
POST-THAW CELL COUNT PREDICTS ENgraftMENT RATE IN CORD BLOOD TRANSPLANTATION

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Background: The infused cell count in cord blood transplantation (CBT) is an important element for engraftment; however, the number in the prior reports has been based on the pre-thaw cell count. Therefore, the association between post-thaw cell count and engraftment rate, especially in pediatric patients, is unclear.

Aims: The aim of this study is to reveal the association between post-thaw cell count and engraftment rate in pediatric patients in the setting of CBT at our institution.

Methods: We retrospectively reviewed the medical records of 78 patients who underwent CBT between June 1998 and April 2016. We excluded the cases of CBT that required rescuing after engraftment failure.

Results: Underlying disease was acute leukemia (AL) in 63 (ALL: 38; AML: 25) patients, chronic myeloid leukemia in one, malignant lymphoma (ML) in two, myelodysplastic syndrome (MDS) in three, aplastic anemia in one, and others (such as primary immunodeficiency syndrome) in eight. In terms of conditioning regimens, myeloablative conditioning was administered to 62 patients and reduced intensity conditioning was administered to 16 patients. The median age at CBT was 3 (range: 0–19) years, and the median follow-up period was 898 (range: 47–6236) days. The engraftment rate was 84.6%, primary engraftment failure was observed in 11 patients (AL:seven; ML: one; MDS: one; neutroblatoma, one; and others, one) and secondary graft failure was observed in one patient. The median times to neutrophil recovery curves were 12 days (sever central neutrophils) and 14 days. Secondary failure rate was 55.1%, and 32 patients had died (cause of death: progressive disease in 19 patients). We analyzed the data on 34 patients of whom both of pre- and post-thaw CD34+ cell counts in the cord blood samples were available. The median post-thaw CD34+ cell count was 1.60 × 10^5/kg in the patients who achieved engraftment and 1.01 × 10^5/kg in the patients who did not achieve engraftment. No statistically significant difference was observed between these two groups (p=0.30). When we defined the cut-off value of the pre-thaw CD34+ cell count as 1.2 × 10^5/kg in the patients who were infused with CD34+ cells more than the cut-off value, the specificity and sensitivity of graft failure was 79.3% and 60%, respectively. When we defined the cut-off value of the post-thaw CD34+ cell count as 0.7 × 10^5/kg in the patients who were infused with CD34+ cells more than the cut-off value, the specificity and sensitivity of graft failure was 96.6% and 40%, respectively.

Summary/Conclusions: We concluded that the risk of graft failure is more precisely predicted by the post-thaw than pre-thaw CD34+ cell count and that if the post-thaw CD34+ cell count is more than 0.7 × 10^5/kg, the risk of graft failure is very low.

URIC ACID LEVEL MIGHT BE A PROGNOSTIC INDICATOR FOR SURVIVAL IN PATIENTS WHO UNDERWENT ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION. SINGLE CENTER EXPERIENCE

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Background: Uric acid (UA) is an abundant aqueous antioxidant that accounts for almost two thirds of all free-radical-scavenging activity in human serum. It is released from injured cells during conditioning for allogeneic hematopoietic stem cell transplantation (AHSCT).

Aims: The aim of this study was to evaluate the prognostic impact of pre transplantation uric acid levels on survival and mortality in allogeneic HSCT patients.

Methods: We retrospectively analyze 273 patients with hematologic diseases unseating AHSCT. The patients were categorized as patients with acute leukemia, myelodysplastic syndrome, lymphoma patients and other hematologic disease diagnoses. A serum uric acid concentration 3.4 mg/dl was considered hypouricemia. Pretransplantation uric acid, creatine, total protein and albumin were analyzed. Univariate, multivariate Cox regression models and Kaplan-Meier survival curves were performed to uric acid, creatine, total protein and albumin associated with disease-free survival (DFS) over all survival (OS), early non relaps mortality (+30 day) and late non relaps mortality (+100 day).

Results: Pretransplantation low uric acid levels were detected in 57% (52/87) and low UA levels were significantly associated with DFS (HR: 0.52; p=0.027). None of the creatine, total protein and albumin were significantly associated with DFS (HR:0.98; p=0.98, HR:0.87; p=0.60, HR: 1.15; p=0.66). There was no significant association between UA, creatine, total protein and albumin and overall survival (HR: 0.84; p=0.46, HR: 2.10; p=0.07, HR: 0.88; p=0.52, HR: 0.78; p=0.26), early relapse mortality (HR: 1.38; p=0.54, HR: 2.16; p=0.29, HR: 0.61; p=0.25, HR: 0.53; p=0.13) and late non-relapse mortality (HR:0.57; p=0.35, HR: 0.21; p=0.29, HR: 1.04; p=0.94, HR: 1.07; p=0.92).

Summary/Conclusions: Uric acid is a natural antioxidant compound. UA reacts with oxygen-derived free radicals and becomes oxidized. Since humans are unable to catabolize UA to the more soluble compound allantoin due to lack of urate oxidase or uricase, the serum UA concentration is higher in humans than almost all mammals. However, this high UA level in humans has been regarded as being beneficial in the presence of elevated oxidative stress. Our study supports that the uric acid is an antioxidant compound. Uric acid level was a free survivor characteristic in the patients who underwent allogeneic HSCT. This is the first report demonstrating a positive association between UA levels and survival analyses in allogeneic HSCT patients. Our findings are potentially clinically relevant. Confirmation in independent cohorts and further investigations into underlying mechanisms, such as reduced antioxidative capacity in high hypouricemia patients is warranted. The result of increased works on this subject, uric acid may be considered a possible prognostic marker in allogeneic hematopoietic stem cell transplantation.

RISK FACTORS FOR HERPES SIMPLEX VIRUS-1/2 VIREMIA AND CLINICAL OUTCOMES FOLLOWING UNMANIPULATED HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Herpes simplex virus(HSV)-1/2 can still be reactivated after allogeneic hematopoietic stem cell transplantation (allo-HSCT) even when the prophylactic acyclovir is used. However, the risk factors for HSV-1/2 viremia and the clinical outcomes following unmanipulated haploidentical HSCT remain unknown.

Aims: The aim of this study was to explore the risk factors for HSV-1/2 viremia and to evaluate clinical outcomes of following haploidentical HSCT.

Methods: Nineteen patients with HSV-1/2 viremia and fifty-seven patients without HSV-1/2 viremia which were selected using the case-pair method after haploidentical HSCT were enrolled. We analysed the risk factors for HSV-1/2 viremia and compared clinical outcomes between the two patient groups.

Results: The risk factors for HSV-1/2 viremia included HLA disparity ≥2 loci (p=0.049) and cytomegalovirus (CMV) reactivation (p=0.028). The incidences of platelet engraftment, oral mucositis and severe haemorrhagic cystitis (HC) in patients with and without HSV-1/2 viremia were 77% and 94% (p=0.003), respectively.
78% and 13% (p=0.000), and 25% and 6% (p=0.04), respectively. Moreover, the median time to platelet engraftment in patients with and without HSV-1/2 viremia was 25 d (range, 11–80 d) and 17 d (range, 8–67 d) (p=0.004). In a multivariate analysis, HSV-1/2 viremia was associated with delayed platelet engraftment (p=0.038), a higher incidence of oral mucoctis (p=0.000) and severe HC (p=0.038). However, HSV-1/2 viremia was not associated with non-relapse mortality (3-year OS vs 31.5%, p=0.26) or leukemia-free survival (60.9% vs 57.9%, p=0.46) and overall survival (61.2% vs 60.7%, p=0.37) (Figure 1).

Figure 1.

Summary/Conclusions: Based on our study results, we recommend that HSV-1/2 PCR should be performed on clinical suspicion.

PB2173
FACTORS PREDICTING GRAFT-VERSUS-HOST DISEASE-FREE, RELAPSE-FREE SURVIVAL AND OUTCOMES AFTER REDUCED-INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION FOR ACUTE LEUKAEMIA OR MEYOELDYSPLASTIC SYNDROMES
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Background: Reduced intensity allogeneic stem cell transplantation (RIST) is now commonly applied for elderly patients with acute leukemia (AL) or myelodysplastic syndromes (MDS). However, the factors affecting graft-versus-host disease-free, relapse-free survival (GRFS) and overall survival (OS) remain obscure.

Aims: To identify such factors and to clarify the clinical significance of RIST with various graft sources, we retrospectively analyzed patients with AL or MDS who received RIST in our hospital.

Methods: The study included patients with acute myeloid leukemia (n=73), acute lymphoid leukemia (n=31) or MDS (n=25), who received fludarabine (Flu)/melphalan (Mel)-based RIST between 2004 and 2015 as the first transplantation.

Results: There were a total of 129 patients, including 3 in low risk (L), 74 in intermediate risk (I), 36 in high risk (H) and 13 in very high risk (V), classified by the refined disease risk index (rDRI). The median age was 58 years (range: 18-83 years), with 73 males and 56 females. Conditioning regimens contained Flu (125mg/m²) combined with Mel (80mg/m², n=21 or 140mg/m², n=10). Total body irradiation (4Gy) was used in 96 patients who received transplantation from unrelated donors or HLA mismatched related donors. Bone marrow (BM) or peripheral blood stem cell (PB) from related donors was used in 40 patients, BM or PB from unrelated donors in 33 and cord blood (CB) from unrelated donors in 56. Primary graft failure occurred in 7 patients and death before engraftment was observed in two. After a median follow-up of 46 months (range: 15-144 months) for the survivors, the 1-year GRFS, disease free survival (DFS) and OS were 57%, 61% and 70%, respectively. On univariate analysis for all patients, pre-transplant factors associated with the 5-year GRFS included stem cell (BM/PB vs CB: 44% vs 68%, p=0.005), donors (related vs unrelated: 38% vs 62%, p=0.012), disease (AL vs MDS: 60% vs 28%, p<0.001) and rDRI (L/I vs H/V: 65% vs 38%, p=0.003). On multivariate analysis, BM/PB (HR 2.0, 95% CI 1.0-4.0, p=0.039), MDS (HR 2.6, 95% CI 1.5-4.8, p=0.001) and H/V vs rDRI (HR 2.1, 95% CI 1.2-3.5, p=0.006) were associated with a worse GRFS. The 5-year OS, cumulative incidence of relapse (CIR) and non-relapse mortality (NRM) were 55%, 36% and 18%, respectively. On univariate analysis, significant prognostic factors were hematopoietic stem cell transplantation-specific comorbidity index (HCT-CI) score 0 vs >=1: 78% vs 48%, p=0.007, disease (AL vs MDS: 59% vs 40%, p=0.004) and rDRI (L/I vs H/V: 64% vs 43%, p=0.003) for the 5-year OS, donors (related vs unrelated: 53% vs 27%, p=0.005) and rDRI (L/I vs H/V: 27% vs 48%, p=0.005) for CIR, and age (<60 vs >= 60: 10% vs 28%, p=0.021), donors (related vs unrelated: 8% vs 23%, p=0.034) and disease (AL vs MDS: 13% vs 36%, p=0.003) for NRM. On multivariate analysis, HCT-CI score (HR 1.9, 95% CI 1.3-3.4, p=0.005) were adversely associated with OS, so were H/V vs rDRI (HR 2.5, 95%CI 1.4-4.7, p=0.003) and MDS (HR 3.7, 95%CI 1.6-8.8, p=0.002) for CIR and NRM, respectively.

Summary/Conclusions: Our data suggest that Flu/Mel-based RIST is a promising strategy for patients with hematologic malignancy, irrespective of (?) donor or stem cell sources. However, GRFS and OS of MDS were significantly worse than those of AL, and MDS is strongly associated with high NRM even with RIST. This indicates that we should pay more attention to NRM in MDS.

PB2174
INCIDENCE AND RISK FACTORS FOR THE DEVELOPMENT OF HEMORRHAGIC CYSTITIS ON HAPLOIDENTICAL TRANSPLANTATION
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Background: Hemorrhagic cystitis (HC) is a serious complication occurring after allogeneic hematopoietic stem cell transplantation (HSCT) more frequent on haploidential (haplo) HSCT, with an incidence of 10% to 70% (Silva et al Haematologica 2010;95(7):1183–1190) associated mainly with the effect of cytotoxic agents such as Cyclophosphamide (Cy). The conditioning regimen, BKPyV infection and graft versus host disease have an implication in the incidence. Other authors related the reactivation of CMV and a previous transplantation as risk factors to HC development (Ruggeri et al Transplant Infectious Disease 2015;17:822–830).

Aims: With this study we aim to describe the HC incidence and risk factors in all haplo-HSCT performed in the Canary Islands.

Methods: We analyzed all consecutive haplo-HSCT from family donors performed at our Hospital between 2013 and 2016. The conditioning regimens used for the transplant was the Hopkins haplo protocol with high dose Cy (50 mg/kg on days 3 and 4) posttransplantation (PTCy). We used as HC prophylaxis intense hydration on the Cy administration day and the following 24 hours (using bladder wash only in 1 patient with cardiac dysfunction) and perfused MESNA at 100% of Cy dose beginning 15 minutes before the Cy administration on 16 pts and at 20% of the last dose at 0, 4 and 8 hours on all pts. We used SPSS V.23 to determine the cumulative incidence (CI) of HC.

Results: We performed 20 haplo-HSCT, of which 10 were males (1 was transplanted 3 times) and 8 were women. The mean age was 40 (range 16-64). The pts presented the following diagnosis: AML (10), ALL (1), EH (5), NHL (3), AM (1). 45% of pts received the haplo-HSCT in remission, 50% with refractory disease and 5% of pts did not receive previous treatment. 6 pts developed HC (36.5% CI at day +80) (Figure 1a) with a median time from haplo-HSCT to onset of 23 days (range 3-42), 1 (17%) was grade I, 4 (66%) grade II and 1 (17%) grade IV. The grade I case did not receive the MESNA infusion like most of the other pts. No pts died due to HC and all cases resolved without sequelae. 12 pts received Cy pre- and post-transplant and only 8 pts received PTCy. The CI at day +80 for the pts with PTCy was 33.3% and for Cy pre- and post-transplant 38.3% (Figure 1b). We found no statistically significant difference on the CI of HC between these two groups. The development of HC was related to Cy in 1 patient, who suffered from this complication on the second and third haplo-HSCT. For the rest of the pts (after day +30) the HC was related to BKPyV infection, as a consequence of the immunosuppression state of the patient, we also observed all these pts had positive serum viral load for CMV.

Summary/Conclusions: The incidence of HC associated to post-HSCT high Cy dose in our series is 15% lower than other ones. Most of them on grade 1 or 2 and without mortality associated. The risk of HC is high, particularly in the setting of highly pre-treated patients (especially those undergoing a 2nd transplant). The development of HC after day +30 is evidently associated to BKPyV as a contributing factor for continuous inflammation and CMV reactivation (as an immunosuppression marker). In our study, HC did not have an impact on the survival or quality of high-risk patients after haplo-HSCT. The HC remains frequent with a high morbidity in particular when it is severe, often causing prolonged hospitalization and resource use. We need further studies to recognize the at-risk population early.
Background: Although the Bruton tyrosine kinase (BTK) inhibitor ibrutinib significantly improves the prognosis of CLL patients (pts), allogeneic hematopoietic stem cell transplantation (HCT) remains the only curative option for the underlying disease. Data on pre-transplant treatment of CLL with ibrutinib are very limited.

Aims: Here we present our experience of HCT in pts previously treated with ibrutinib.

Methods: 11 CLL pts (median age at HCT 57 years [y], range 52-66 y) treated between 2014 and 2016 in our unit with non-myeloablative (nma) HCT after ibrutinib were included. Ibrutinib treatment lasted median 4.03 months (range 1-28). Conditioning regimen was Flu达尔bin 30 mg/m² on day -4 to -2 followed by 2 Gy total body irradiation. Disease status at HCT was Binet B (n=3) or Binet C (n=8). Two pts had Richter’s transformation (RT) diagnosed before nma-HCT. Ten pts were in partial remission (PR) at nma-HCT (PR1:n=4; PR2:n=3; PR3:n=2, PR4:n=1) while one was in first relapse. Donors were human leukocyte antigen (HLA) matched related (n=3, MRD) or HLA-matched unrelated (n=8, MUD). Pts received median 3 lines of therapy (range 1-6) including ibrutinib before transplantation. Classical cytogenetic analysis and fluorescence in situ hybridization (FISH) was carried out for every pt. Five pts had a deletion (del)(17p13) and one a del(11q22.3).

Results: The average overall survival (OS) for all pts was 471 days (range 36-812) (Figure 1). The average OS of patients with del(17p13) was 379 days (range 66-628) compared to 456 days (range 36-812) for those without del(17p13, p=0.98). OS was not significantly influenced by the stem cell source (MUD vs MRD, p=0.63) or remission status PR1 vs >PR1 (353 vs 472 days, p=0.79). Non-matched CMV-Status (negative recipient and positive donor or positive recipient and negative donor) had an OS comparable to that of matched CMV-Status (p=0.73). Pts above the median age had a lower OS although this didn’t reach significance (p=0.39). EFS was median 125 days (range 26-628). Pts with or without a TP53 alteration had a similar EFS (p=0.91). Pts undergoing MRD-HCT had better EFS than those undergoing MUD transplantation (p=0.055). CMV-Status or age>median had no prognostic influence on the EFS (p=0.83 and p=0.39 respectively). Non-relapse mortality (NRM) was 32% at 10 months (Figure 1), which was consistent with a previous publication from our group (30% at 4y). The acute GvHD Grade 3-4 was present in 3 pts (27.2%).

Summary/Conclusions: Impact of chimerism in different T-helper subsets still need further investigation. We will continue our research and further results will be reported later.

Background: Despite the fact that almost all studies in transplant biology dedicate T-cells the chimerism in T-helper (Th) cells and its subsets such as T-regulatory (Treg) cells after allogeneic hematopoietic stem cell transplantation (allo-HSCT) has never been evaluated.

Aims: To evaluate Th, Treg and bone marrow cell short-term chimerism in allo-HSCT patients.

Methods: Between May 2015 and November 2016 there was 109 transplants in our center. The research included 24 patients with hematological malignancies (AML =14, ALL =7, MDS =2, CMML =1). The median age of patients was 33.5 (range 19 to 60) years, male=16, female=8. Myeloablative conditioning regimen was used for 11 pts. The other 13 pts underwent non reduced intensity conditioning regimen. Peripheral blood stem cells (PBSCs) as graft source was used in 9 patients, BM in 15 patients. 9 patients were transplanted from HLA-identical related donor, 15 - from unrelated matched donor. Сhimerism source was used in 9 patients, BM in 15 patients. 9 patients were transplanted from HLA-identical related donor, 15 - from unrelated matched donor. Сhimerism source was used in 9 patients, BM in 15 patients. 9 patients were transplanted from HLA-identical related donor, 15 - from unrelated matched donor. Сhimerism source was used in 9 patients, BM in 15 patients. 9 patients were transplanted from HLA-identical related donor, 15 - from unrelated matched donor. Сhimerism source was used in 9 patients, BM in 15 patients. 9 patients were transplanted from HLA-identical related donor, 15 - from unrelated matched donor. Сhimerism source was used in 9 patients, BM in 15 patients. 9 patients were transplanted from HLA-identical related donor, 15 - from unrelated matched donor. Сhimerism source was used in 9 patients, BM in 15 patients. 9 patients were transplanted from HLA-identical related donor, 15 - from unrelated matched donor. Сhimerism source was used in 9 patients, BM in 15 patients. 9 patients were transplanted from HLA-identical related donor, 15 - from unrelated matched donor. Сhimerism source was used in 9 patients, BM in 15 patients. 9 patients were transplanted from HLA-identical related donor, 15 - from unrelated matched donor. Сhimerism source was used in 9 patients, BM in 15 patients. 9 patients were transplanted from HLA-identical related donor, 15 - from unrelated matched donor. Сhimerism source was used in 9 patients, BM in 15 patients. 9 patients were transplanted from HLA-identical related donor, 15 - from unrelated matched donor. Сhimerism source was used in 9 patients, BM in 15 patients. 9 patients were transplanted from HLA-identical related donor, 15 - from unrelated matched donor. Сhimerism source was used in 9 patients, BM in 15 patients. 9 patients were transplanted from HLA-identical related donor, 15 - from unrelated matched donor. Сhimerism source was used in 9 patients, BM in 15 patients. 9 patients were transplanted from HLA-identical related donor, 15 - from unrelated matched donor. Сhimerism source was used in 9 patients, BM in 15 patients. 9 patients were transplanted from HLA-identical related don
PB2178

NON RELAPSE MORTALITY (NRM) ANALYSIS IN 93 UNRELATED DONOR TRANSPLANTATION - SINGLE CENTRE EXPERIENCE - HLA HAPLOTYPE ROLE?

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Background: Unrelated donor stem cell transplantation has a curative potential against haematological malignancies. However there are concerns about associated risk of non-relapse mortality. We performed a retrospective single centre study of causes of non-relapse mortality over four year period - 2012-2016.

Aims: Purpose of the study was to analyse non-relapse mortality (NRM) in patients subjected to unrelated donor transplantation in four-year-period: 2012 to 2016 - 93 transplant procedures in 86 patients.

Methods: Study cohort was analysed - relapse rate and non-relapse mortality were assessed. Causes of both - early and late NRM were studied.

Results: There were 23 relapses in the group of assessed patient cohort (24,7%). 7 patients undergone the second transplant - five patients - because of AML relapse, TAT - 5 patients with graft failure. Out of 16 transplanted 7 patients - 3 patients are alive - 2 patients with graft failure and one with post-transplant AML relapse in 2nd CR. Out of 93 procedures of unrelated donor transplantation there were 16 cases of death - assumed as non relapse mortality NRM (17%). There were 9 early deaths (before day +100) - 6 cases in patients with relapsed/refractory acute leukaemia without remission after conventional chemotherapy. These patients were subjected to sequential conditioning with cytreduction phase. Active disease and highly active antileukaemic treatment can be reason for higher treatment related toxicity and elevated risk of death. Later two patients developed infectious bacterial complications with septic shock. In one patient - antiviral treatment refractory CMV encephalitis with massive macrophage activation syndrome was diagnosed. Analysis of NRM after day100 revealed 7 affected patients. All these patients GVHD 2-4 was diagnosed previously, accompanied by transplant associated microangiopathy (TAM) and infections - both viral and fungal. Additionally to factors connected to NRM - age, comorbidity score, patient/donor HLA allelic and antigen and sex mismatches, HLA patient/donor haplotypes were analysed. It was possible to categorise 15 out of 16 NRM patients into 5 HLA class II haplotype groups connected with autoimmune diseases in Caucasian population - rheumatoid arthritis and lupus erythematosus: DRB1 01:01 DQB1 05:01 (6 patients), DRB1 03:01 DQB1 02:01 (4 patients), DRB1 11:01 DQB1 03:01 (3 patients), DRB1 15:01 DQB1 06:02 (2 patients), DRB1 04:01 DQB1 03:02 (1 patient).

Summary/Conclusions: Based on these results we create working hypothesis that HLA class II haplotype may predispose to severe post-transplant infectious or/and non-infectious complications and affect the risk of NRM. Because small number of analysed patients and documented high frequency of these haplotypes in population, further analysis is required.

PB2179

HAPLOIDENTICAL STEM CELL TRANSPLANTATION WITH HIGH DOSE CYCLOPHOSPHAMIDE POST-TRANSPLANT IN HIGH RISK HEMATOLOGIC MALIGNANCIES: RISK FACTOR AND OUTCOME ANALYSES IN OUR CENTER

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Background: Allogenic hematopoietic stem cell transplantation (allo-HSCT) is an effective therapy for a variety of hematological malignancies. However, a lack of HLA-identical sibling donors or unrelated donors has restricted the application of allo-HSCT in hematological malignancies. Haploidentical HSCT (Haplo-HSCT) offers the benefits of rapid and nearly universal donor availability and, in the past decade, has been accepted worldwide as an alternative treatment for patients with hematological malignancies who do not have an HLA-identical sibling donor or who require urgent transplantation.

Aims: The purpose of this study was to investigate the incidence, causes and factors influencing overall and transplant-related mortality after Haplo-HSCT.

Methods: We analyzed all consecutive patients receiving Haplo-HSCT from family donors at our hospital from 2013 to 2016. The conditioning regimen used for the transplant was the Hopkins haplo protocol with high dose Cy (50 mg/kg on days 3 and 4) posttransplantation. We classified the patients before the Haplo-HSCT according to disease risk index (DRI), ECOG, Sorror score and EBMT risk score to evaluate the correlation between the physical state of the patients before the transplant and the survival (overall mortality (OM) and transplant-related mortality (TRM)). We used SPSS V 23 to calculate the cumulative Mortality incidence by the KM test and the Cox proportional hazards model.

Results: We performed 20 haplo-HSCT, 10 were males (1 was transplanted 3 times) and 8 were females mean age of 40 (range 16-64). Diagnosis: AML (10), ALL (1), EH (5), NHL (3), AM (1). Forty five percent of patients received the haplo-HSCT in remission, 50% with refractory disease and 5% of patients did not receive previous treatment. Of the 20 patients from our series, 12 died post transplant with an OM of 60%. The cumulative incidence (CI) of OM was 15% at 1 month (m), 35% at 3 m, 45% at 6 m, 55% at 1 year, and 40% at 2 and 3 years (Figure 1a). When we analyzed the OM depending on the different physical status scores we found no statistically significant differ-
Summary/Conclusions: The long-term stability of cryopreserved peripheral blood stem cells (PBSCs) is an important concern for patients experiencing disease relapse. However, the quality of long-term cryopreserved PBSCs stored at -80°C by using simplified method has not been elucidated in detail. Cryopreserved PBSCs undergo cell damage and decrease in viability, and those containing granulocytes might influence cell loss.

Aims: The aim of this study was to evaluate the effect of cryopreservation for less than 36 months and the number of granulocytes in the cryopreserved PBSC products on CD34+ cells.

Methods: We examined the effects of cryopreservation on the viability of CD34+ cells that were stored for less than six months and those stored for 7–24 months, and 25–36 months, and the change of CD34+ cell viability with higher granulocyte content. We also evaluated the correlations between the number of granulocytes in the cryopreserved PBSC products and the time to engraftment of lymphocyte or platelet. Informed consent was obtained prior to the procedure from all the patients following institutional guidelines.

Results: A total of 65 PBSC samples were collected. We compared three groups based on the cryopreservation period: (1) less than 6 months, (2) 7–24 months, and (3) 25–36 months. The median (range) viability of CD34+ cells after thawing was 81.8% (59.2–94.4), 80.5% (56.6–92.8), and 76.1% (54.5–89.6) in the three groups, respectively. No significant difference in the viability of the cells in either frozen period was observed (p=0.14, respectively). We compared the effect of granulocyte concentration (over 10% concentration against less than 10% concentration) on CD34+ cells viability. The median (range) viability of CD34+ cells containing >10% granulocytes was 76.6% (54.5–93.0%), and that for cells containing <10% granulocyte was 82.1% (59.1–94.4%), respectively. There was significant difference in the viability of CD34+ cells between the two groups (p=0.02, respectively). Second, we analyzed 81 autologous PBSC transplants after stored at -80°C by using simplified method. We compared two groups based on the granulocyte concentration (>10% concentration against <10% concentration). No significant difference in the days to leukocyte >1x10^9/L and to platelet >20x10^9/L in either granulocyte concentration was observed. However, the median (range) time to platelet >50x10^9/L containing >10% granulocytes was 27.2 (12–87), and that for cells containing <10% granulocyte was 20.3 (10–51), respectively. There was significant difference in the day to platelet >50x10^9/L between the two groups (p=0.04, respectively).

Summary/Conclusions: A delay in lymphocyte recovery is associated with a decrease in survival rates in our patients. Measures favoring an accelerated lymphocyte recovery (prophylactic use of thymoglobulin, adequate donor selection, and transplantation modality) could affect the post-transplant survival. It appears that the amount of infused product could play an important role in reinstatement, so it would be a factor to take into account prior to infusion.

PBZ2180
A SIMPLIFIED METHOD OF CRYOPRESERVATION OF PERIPHERAL BLOOD STEM CELLS WITH OVER 10% GRANULOCYTE CONCENTRATION FOR LESS THAN 36 MONTHS
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Background: The long-term stability of cryopreserved peripheral blood stem cells (PBSCs) is an important concern for patients experiencing disease relapse. However, the quality of long-term cryopreserved PBSCs stored at -80°C by using simplified method has not been elucidated in detail. Cryopreserved PBSCs undergo cell damage and decrease in viability, and those containing granulocytes might influence cell loss.

Aims: The aim of this study was to evaluate the effect of cryopreservation for less than 36 months and the number of granulocytes in the cryopreserved PBSC products on CD34+ cells.

Methods: We examined the effects of cryopreservation on the viability of CD34+ cells that were stored for less than six months and those stored for 7–24 months, and 25–36 months, and the change of CD34+ cell viability with higher granulocyte content. We also evaluated the correlations between the number of granulocytes in the cryopreserved PBSC products and the time to engraftment of lymphocyte or platelet. Informed consent was obtained prior to the procedure from all the patients following institutional guidelines.

Results: A total of 65 PBSC samples were collected. We compared three groups based on the cryopreservation period: (1) less than 6 months, (2) 7–24 months, and (3) 25–36 months. The median (range) viability of CD34+ cells after thawing was 81.8% (59.2–94.4), 80.5% (56.6–92.8), and 76.1% (54.5–89.6) in the three groups, respectively. No significant difference in the viability of the cells in either frozen period was observed (p=0.14, respectively). We compared the effect of granulocyte concentration (over 10% concentration against less than 10% concentration) on CD34+ cells viability. The median (range) viability of CD34+ cells containing >10% granulocytes was 76.6% (54.5–93.0%), and that for cells containing <10% granulocyte was 82.1% (59.1–94.4%), respectively. There was significant difference in the viability of CD34+ cells between the two groups (p=0.02, respectively). Second, we analyzed 81 autologous PBSC transplants after stored at -80°C by using simplified method. We compared two groups based on the granulocyte concentration (>10% concentration against <10% concentration). No significant difference in the days to leukocyte >1x10^9/L and to platelet >20x10^9/L in either granulocyte concentration was observed. However, the median (range) time to platelet >50x10^9/L containing >10% granulocytes was 27.2 (12–87), and that for cells containing <10% granulocyte was 20.3 (10–51), respectively. There was significant difference in the day to platelet >50x10^9/L between the two groups (p=0.04, respectively).

Summary/Conclusions: A delay in lymphocyte recovery is associated with a decrease in survival rates in our patients. Measures favoring an accelerated lymphocyte recovery (prophylactic use of thymoglobulin, adequate donor selection, and transplantation modality) could affect the post-transplant survival. It appears that the amount of infused product could play an important role in reinstatement, so it would be a factor to take into account prior to infusion.
PB2182

SUCCESSFUL AUTOLOGOUS STEM CELL TRANSPLANTATION AFTER VELCADE-BASED REFRACTORY MULTIPLE MYELOMA PATIENTS

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Background: The optimal induction treatment for Newly Diagnosed Multiple Myeloma Patients needs combinations with Bortezomib-Based (Bor-based) schemes. Primary refractory patients include patients with progressive disease or rapid (<60 d) relapse after these optimal induction approach have a very bad prognosis. Lenalidomide-Dexamethasone (LenDex) were usually the next step in the treatment of these patients, until the recent introduction of triplets combination LenDex-based. Autologous Stem Cell Transplantation (ASCT) has proven efficacy in NDMM younger patients that have got at least a partial response (PR) after the induction therapy. There are few data about toxicity and response of ASCT in primary refractory patient that can obtain a response with LenDex rescue treatment.

Aims: Analysis of tolerance, response and overall survival of ASCT-candidates that are primary refractory to Bor-Based induction treatment.

Methods: Retrospective analysis of our database. From 2010 to Nov-2016, 53 ASCT-Candidates (for 1st or 2nd ASCT procedures) were included. Median Age for diagnosis was 62 (46-71). Median Age for ASCT procedure was 63 (46-72). 12 of these 53 patients (22.6%) were considered primary refractory and considered candidates to get 2nd Based treatment. 6 of them (50%) were woman. Characteristics of Disease: IgG kappa (4), IgG-lambda (3), IgA kappa (3), IgA lambda (1), Light Chain lambda (1). ISS I/II/III: 5/2/5. Induction treatment: VelDex (4), VTD (6), VCD (2). Median of cycles administered: 6 (2-8). Best Response to induction treatment: >PR (6), Minimal Response (1), progressive disease (1), non evaluable (1).

Results: Median number of cycles administered: 6 (3-12). Of them didn’t respond. Of the other 9, 6 of them were considered candidate to intensificate treatment with high doses chemotherapy supported with an ASCT (2 of 6 to a 2nd ASCT procedure). The 3 other patients are in treatment or preASCT evaluation.

Summary/Conclusions: Our results show that This TBF conditioning regimen appears to be safe,allows high rate of engraftment and low NRM rate among high-risk patients and can lead to a long-term disease control.

PB2183

SAFETY AND EFFICACY OF TBF CONDITIONING IN PATIENTS UNDERGOING ALLOGENEIC STEM CELL TRANSPLANTATION.A RETROSPECTIVE SINGLE CENTER EXPERIENCE.

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Background: The optimal intensity of myeloablation with a reduced-toxicity conditioning (RTC) regimen to decrease relapse rate after allogeneic stem cell transplant (allo-SCT) without increasing non-relapse mortality (NRM), has not been well established.

Aims: In this retrospective study at the American University of Beirut medical center (AUBMC) we aimed to evaluate the outcomes of patients who underwent allo-SCT with thiopeta, busulfan and fludarabine (TBF) as RTC.

Methods: We included twenty four consecutive patients with hematological malignancies who received TBF as conditioning for allo-SCT from January to December 2016. All patients and transplant characteristics are listed in Table 1. All patients received the myeloablative conditioning regimen consisting of thiopeta (5mg/kg/day) infused on day -7 and -6, fludarabine (30mg/m2/day) infused on days -5 to -2 and busulfan (130mg/m2/day) was infused on days -5 to -2. All patients received 2.5mg/kg/day intravenous rabbit antithymocyte globulin (ATG) on days -2 and -1. GVHD prophylaxis for patients transplanted from haploidentical donor consisted of post-transplant cyclophosphamide 50mg/kg/day on day +3 and +5, cyclosporine started at 3 mg/kg/day on day +6 and readjusted according to level, and mycophenolate mofetil 500mgx4/day started on day +6 to +28. Patients transplanted from matched unrelated donor, received cyclosporine as of day +1. Patients with NRM were mainly treated with R-CHOP/R-CVP (82.5%) at first-line, or rapid (<60 d) relapse after these optimal induction approach have a very bad prognosis. Lenalidomide-Dexamethasone (LenDex) on days -2 and -1. GVHD prophylaxis for patients transplanted from haploidentical donor consisted of post-transplant cyclophosphamide 50mg/kg/day on day +3 and +5, cyclosporine started at 3 mg/kg/day on day +6 and readjusted according to level, and mycophenolate mofetil 500mgx4/day started on day +6 to +28. Patients transplanted from matched unrelated donor, received cyclosporine as of day +1.

Results: Twenty three patients (96%) engrafted, with 14 days (range, 10-18) and 13 days (range, 8-48) as median time for neutrophil and platelet engraftment respectively. One patient who underwent haploidentical donor transplant with persistent disease for AML (karyotype 45,XY,-7) failed to engraft and died due to disease progression on day +22. After a median follow up of 10 months (range, 1-22) post-allo-SCT, the cumulative incidence of Gradel-I acute GVHD (aGVHD) was 26%. One patient developed chronic limited GVHD (cGVHD). All the complication post allo-SCT are listed in Table 1. Five patients (24%) relapsed post allo-SCT at a median of 163 days (range, 55-384), of whom 4 (80%) died. Two patients (4%) died due to disease progression and two were successfully salvaged and are in complete remission (CR) with full donor chimerism (FDC) at last follow up. Two patients developed JC virus progressive multifocal leukoencephalopathy, one of them made a full recovery and the other died in CR. The day 100 NRM was 0%. At last follow up 20 patients (83%) are alive in CR, with negative minimal residual disease and FDC.

Table 1.

Summary/Conclusions: Our results show that This TBF conditioning regimen appears to be safe,allows high rate of engraftment and low NRM rate among high-risk patients and can lead to a long-term disease control.

PB2184

COMPLETE REMISSION STATUS BEFORE AUTOLOGOUS STEM CELL TRANSPLANTATION AS PROGNOSTIC FACTOR IN PATIENTS WITH NON-HODGKIN LYMPHOMA

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Background: High dose chemotherapy (HDC) followed by autologous stem cell transplantation (ASCT) is commonly used for treatment of relapsed or refractory non-Hodgkin’s lymphoma (NHL), as well as for first-remission consolidation in patients with mantle cell lymphoma. Disease status before ASCT is variable and is unclear whether complete response before ASCT or after ASCT correlates with better survival.

Aims: To evaluate the prognostic effect of disease status before ASCT - complete remission (CR) vs partial remission (PR) - in a cohort of patients with NHL.

Methods: Retrospective analysis of patients with NHL treated with HDC and ASCT between 2007 and 2016 in a single institution. All patients received peripheral blood cell support after conditioning with BEAM regimen (carmustine 300mg/m2, etoposide 800mg/m2, Ara-c 1600mg/m2 and melphalan140 mg/m2). Response was assessed according to The Lugano Classification. The Kaplan-Meier method was used to estimate progression free survival (PFS) and overall survival (OS) and comparison between risk groups was performed by using the log-rank test. For those who did not achieve a CR or relapsed after first-line treatment, (R)-ESHAP/DAHAP/CICE (78.8%) was performed as second-line followed by ASCT as salvage therapy in order to achieve and consolidate CR. The majority of patients with mantle cell lymphoma received R-CHOP/R-DHAP (55.0%) followed by consolidation with ASCT in first remission. With a median follow-up time from ASCT of 39.66 months (0.3-117.6), OS at 2 and 5 years was 84.8%
and 74.5% and PFS was 76.8% and 58.2%, respectively. Before ASCT, 60 patients (72.3%) were in CR and 23 (27.7%) were in PR. After ACST, 4 patients were not assessed for response due to early death by toxicity. Of the remaining, 70 (86.6%) achieved a CR, 4 (5.1%) a PR and 5 (6.3%) failed to respond. Patients in CR before ASCT presented significantly longer PFS compared with those in PR (107.9 vs 44.0 months, p=0.01). Besides that, patients that obtained CR after ASCT also had longer OS and PFS compared with those in PR (107.9 vs 8.0 and 107.9 vs 7.3 months, p<0.001). However, these patients had significantly lower PFS compared to patients that continued in CR after ASCT (45.3 vs 107.9 months, p=0.041). Univariate analysis indicated that remission status prior to ASCT (CR vs PR) is a significant predictor of PFS after ASCT (HR 0.39; 95% CI 0.19-0.82, p=0.013). Multivariate Cox regression model showed that this factor retains prognostic value after adjustment for age, histological subtype, Ann Arbor stage and number of previous lines of treatment.

Summary/Conclusions: Our results highlight the relevance of the obtained CR after ASCT in the OS. Furthermore, we conclude that patients with NHL who are in CR before ASCT have a better PFS than those in PR before ASCT. Additionally, continued CR after ASCT may also be an important prognostic factor. Our results suggest that the use of more effective induction regimens in order to improve initial response may be advantageous in terms of clinical benefits post-ASCT.

PB2185
AUTOLOGOUS STEM CELL TRANSPLANTATION FOR MANTLE CELL LYMPHOMA: SINGLE CENTER EXPERIENCE
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Background: Mantle cell lymphoma accounts for relatively small proportion (3%-10%) of non-Hodgkin lymphoma. High-dose chemotherapy (HDT) and autologous-stem cell transplantation (ASCT) has played a critical role in the treatment of mantle cell lymphoma. Regardless of that, mantle cell lymphoma remains largely a relapsing/remitting disease.

Aims: Our aim is to present our mantle cell lymphoma patients who underwent ASCT.

Methods: We retrospectively evaluated our 21 mantle cell NHL patients. The patients were followed after ASCT for relapse.

Results: Patients were followed by a median time of 56.9 months (range, 6-170 months). The median age at diagnosis was 45 (range, 18-69), female to male ratio=5/16. The stages and MIPI scores at diagnosis were as follows: 5% stage I, 19% stage III, 76% stage IV; Low MIPI 29%, intermediate MIPI 48% and high MIPI 23%. First line treatments were R-CHOP for 6 cycles in 6 patients (29%) and R-CHOP for 3 cycles followed by R-DHAP in 15 patients (71%). The median time to ASCT was 20 months (range, 7-45 months). All patients were in at least partial remission at the time of ASCT. The transplant conditioning regimen was CBV in 5 patients (24%) and R-I/ICE in 5 patients (24%), R-I/BEAM in 11 patients (52%). Six patients (29%) achieved complete remission. Four patients (19%) died within three months of ASCT due to infection. Eleven patients (52%) was relapsed with a median time of 39 months (range, 4-123 months). Ten patients received BORID (bortezomib, rituximab, dexamethasone) and 1 patient received lenalidomide as salvage therapy and six of them achieved complete remission. Three patients underwent allogeneic hematopoietic stem cell transplantation as second transplants after the initial relapse, one by matched sibling and one an unrelated cord blood (UCB) transplant; five patients were transplanted from a matched sibling donor (MUD), 5 from a matched unrelated donor (MUD) and 4 from haploidentical donor. Conditioning regimen consisted of busulfan/cyclophosphamide in MUD/MUD patients. The patients transplanted from MUD and UCB also received anti-thymocyte globulin (ATG) for 3–5 days pretransplantation. Haploidentical transplantation was performed with RIC regimen and TCRα/β/CD3 depletion.

Summary/Conclusions: ASCT is a part of initial treatment strategy in fit patients with mantle cell lymphoma however 19 patients in our series had transplant related toxicity. Today, novel agents may present a less intensive treatment option. However, there exist few reports regarding the outcome of transplantation for children with various types of MDS.

PB2187
ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PEDIATRIC MYELODYSPLASTIC SYNDROMES: A SINGLE CENTER EXPERIENCE FROM TURKEY
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Background: Myelodysplastic syndrome (MDS) in childhood is a rare disorder and hematopoietic stem cell transplantation (HSCT) is the only known curative treatment option. However, there exist few reports regarding the outcome of transplantation for children with various types of MDS.

Aims: We analyzed the outcome of pediatric patients who underwent HSCT in our center.

Methods: We reviewed retrospectively 14 pediatric MDS patients who received HSCT at a single center. Median age at time of HSCT of the patients was 4.3 years and disease duration from diagnosis to transplantation ranged from 3 to 36 months with a median of 10 months. Five patients had primary and one had secondary MDS. Four patients had juvenile myelomonocytic leukemia (JMLM) and 4 patients had myelodysplastic related acute myeloid leukemia (MDR-AML). Diagnostic cytogenetics included monosomy 7 (n=2), trisomy 8 (n=3), KRAS (n=1) or normal/other (n=8). Patients received a median of 6.8x10^6 CD34+ cells/kg. Eight patients received a bone marrow, 5 had peripheral blood graft and one an unrelated cord blood (UCB) transplant; five patients were transplanted from a matched sibling donor (MUD), 5 from a matched unrelated donor (MUD) and 4 from haploidentical donor. Conditioning regimen consisted of busulfan/cyclophosphamide in MDS/MUD patients. The patients transplanted from MUD and UCB also received antithymocyte globulin (ATG) for 3–5 days pretransplantation. Haploidentical transplantation was performed with RIC regimen and TCRα/β/CD3 depletion.

Results: Graft failure occurred in three patients with JMLM (n=1), secondary MDS (n=1) and MDR-AML (n=1). Only one patient with MDR-AML underwent second transplantation from another MUD one year after first transplant and died from GVHD. Ten patients are alive with a median follow-up of 19.5 months (range 3-61). All patients with primary MDS are alive and well. Four patients died from transplant-related toxicity (n=2) and relapse (n=2). For the entire group, estimated five-year relapse-free survival (RFS), event-free survival (EFS) and overall survival (OS) were 78.6%, 64.3% and 70.7%, respectively.

Summary/Conclusions: These data demonstrate that especially children with primary MDS may achieve encouraging OS and RFS following HSCT. Relapse remains the main cause of treatment failure in children with JMLM given HSCT. All children with MDS should be referred for allogeneic HSCT soon after diagnosis.
Thalassemias

PB2188

RELATIONSHIP BETWEEN URIC ACID LEVELS AND CARDINAL FINDINGS IN A LARGE COHORT OF β-THALASSEMA MAJOR: GENDER-RELATED DIFFERENCES

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Background: Iron overload, secondary to recurrent transfusions and ineffective erythropoiesis, induces oxidative stress in thalassemia (TM) (Uric acid (UA), a major blood antioxidant, may act either as an antioxidant or pro-oxidant).

Aims: Our aim was to evaluate the role of UA in TM and its association with cardiac iron, dysfunction, fibrosis, and complications, and cardiovascular risk factors in a large cohort of TM patients of both sexes.

Methods: 397 TM patients (200 men, mean age 32±8 years) enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) Network were considered. Myocardial iron and hepatic fibrosis in this study were quantified by the T2* technique. Atrial dimensions and biventricular function were quantified by cine images. Late gadolinium enhancement (LGE) images were acquired to detect myocardial fibrosis.

Results: As expected, UA resulted significantly higher in male respect to female TM patients (4.7±4.1 vs 3.9±0.1 mg/dL; P=0.0001). UA levels directly correlated with BMI (R=0.25, P=0.003), and triglycerides (TG) (R=0.20, P=0.005) in female patients. Moreover, female which presented myocardial fibrosis showed higher levels of UA (4.4±3.1 vs 3.9±0.9 mg/dL; P=0.03). The multiple regression model identified BMI (T-value 3.7, P=0.003), TG (2.1, P=0.04) and cardiac fibrosis (2.5, P=0.01) as independent correlates of UA level in women. In men, UA levels were positively correlated with BMI (R=0.17, P=0.02), TG (R=0.38, P<0.001), and inversely with HDL (R=0.20, P=0.006) and glycemia (R=0.15, P=0.04). Interestingly, UA was also directly correlated with global heart T2* values (R=0.3, P=0.001). After multivariate analysis adjustment, global heart (T-value in 6, P=0.01), T2* (in 7, P<0.001), and BMI (1.9, P=0.05) remained as independent determinants of UA in male TM patients.

Summary/Conclusions: UA levels correlate with factors related to metabolic dysfunction in TM patients of both sex, while a more strong correlation between UA and cardiac fibrosis was observed only in females, and a direct relationship between UA and T2* global heart only in males. The differences in male and female TM patients imply some gender-specific mechanisms, providing biochemical basis for the epidemiological differences between sexes.

PB2189

CHARACTERIZATION OF HEMORHEOLOGICAL ALTERATIONS IN THALASSEMA BY A CHEMOMETRIC APPROACH

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Background: Several studies reported a high incidence of thromboembolic events in β-thalassemia, more frequent in thalassemia intermedia than in regularly transfused thalassemia major. In these patients a chronic hypercoagulable state is evident and the red blood cells exhibit impaired flow properties that facilitate micro-circulatory disorders.

Aims: Since many abnormalities described in thalassemia may determine rheological alterations, in this study we have quantified the T2* technique. The achieved results permit to consider the viscoelastic properties that facilitate micro-circulatory disorders.

Methods: Blood samples from 45 β-thalassemia patients and 48 healthy individuals, after informed consent, were analyzed. Hemorheological profiles were investigated at 37 °C at native and normalized hematocrit. The evaluation of RBCs viscoelastic properties was performed by determining storage modulus G’, loss modulus G’’ and complex modulus G* in oscillation mode as a function of angular frequency ω in the range 0.1–10 Hz. Multivariate statistical analysis was performed on the resulting G’, G” and G* curves and Principal Components Analysis was used as display method.

Results: The hemorheological profiles of patients affected by β-thalassemia and healthy subjects showed significant differences and the chemometric analysis allowed us to perform a clearly identification of anemic status according to viscoelastic profile. Increased G’, G” and G* modula were observed in thalassemic patients demonstrating a reduction in deformability and impaired flow properties.

Summary/Conclusions: In this study a characterization of haemorheological alterations in thalassemia patients has been performed by a chemometric approach. The achieved results permit to consider the viscoelastic properties as promising predictive new indices of microvascular damage in β-thalassemia and to explain the increased incidence of vascular complications in these disorders.

PB2190

HEPATITIS E IN TRANSFUSION-DEPENDENT THALASSEMIA PATIENTS, IN GREECE. A SINGLE CENTER EXPERIENCE

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Background: Hepatitis E (HE) is nowadays considered an emerging disease that may be a threat in both developing and industrialized countries all over the world. The causative agent is an RNA virus, transmitted mainly through the feco-oral route. Nevertheless, there are additional patterns of transmission, including the transfusion of infected blood products. The risk of developing chronic HE infection following transfusion of infected blood–derived products is higher among immune-compromised individuals. Transfusion-dependent thalassemia patients consist a distinct category of immune-compromised patients, but the data regarding transfusion-transmitted HE infection are limited for this group of patients. Accordingly, there is, as yet, no consensus on whether blood products should be systematically screened for markers of the HE virus.

Aims: The aim of this study was to assess the status of Hepatitis E infection in transfusion-dependent Thalassemia patients, followed up in a single Thalassemia Unit, in Northern Greece.

Methods: Over a one-month period, we retrospectively evaluated 96 consecutive patients, from a registry of 150 adult TDT patients followed at a single Thalassemia Unit, in Northern Greece. The mean age of the study population was 51.8±17.5 years. Forty and 56% of the patients were males and females, respectively. According to the patients’ blood transfusion history, the participants had been transfused with 47.376 blood units during the last 14 years, whereas during the last year the patient population had been transfused with 3.384 blood units. The detection of HEV RNA was performed by Real-Time RT-PCR method (hepatitisE2@ceer-Tools kit, Applied Biosystems ABI), according to the instructions. The detection of HEV was based on the identification of the “a” region of ORF2. The detection of IgG anti-HEV antibodies and their titeration were performed in 92/96 samples using a commercially available enzyme-linked immunosorbent assay kit (CUSABIO BIOTECH kit), according to the manufacturer’s instructions.

Results: HE RNA was not detected in any of the 96 samples, whereas the IgG anti-HEV antibodies were also negative in all measured samples. The negative HEV RNA, in all the participants of this study, indicates the absence of an active HE infection, whereas the negative IgG anti-HEV antibody titre implicates that there was no history of previous HE infection. According to the literature, IgG antibodies may be detectable following an HE infection for a time period that varies from one year to 14 years.

Summary/Conclusions: This is the first assessment of the HE virus seroprevalence in the population of TDT patients in Greece, over the last two decades. Our results suggest that TDT patients are not at a high risk for HE infection. Further studies are necessary to evaluate the clinical importance of the transfusion-transmitted HE infection in TDT patients and clarify whether screening of blood donors is necessary for countries with a lower or higher prevalence of HE.

PB2191

Abstract withdrawn.

PB2192

A PRELIMINARY STUDY OF THE CARDIAC EFFECT OF PPAR GAMMA IN IRON OVERLOAD

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1A. ElDefrawy, 2D.D. Elnileey, 3D.O. Ghallab

Background: Peroxisome proliferator-activated receptor (Ppara) agonists in type 2 diabetes patients. Pharmacological agonists of PPAR-gamma leads to a molecular
switch providing alleviating myocardial injury through modulating oxidative, inflammatory and apoptotic signaling pathway.

Aims: Our aim was to investigate the frequency of Pro12Ala polymorphism (substitution of proline to alanine at codon 12 in exon B of PPARγ gene in Egyptian β-thalassemia major (β-TM) with iron overload. Untreated transfusion induced iron overload in thalassemia major is fatal, usually as a result of cardiac complications.

Methods: 30 β-TM patients and 10 healthy volunteer matched for age, sex and body weight were involved in this study. β-TM patients followed up was in the “outpatient clinic of Hematology unit, at Alexandria main university hospital”. Seventeen were males and thirteen were females with ages ranging from 16 – 39 years (21.5±4.4). Blood samples from β-TM patients and healthy controls were analyzed for PPARγ gene polymorphism using polymerase chain reaction-restriction fragment length polymorphism.

Results: The mean value of serum ferritin in β-TM was 4976.30±2216.41 ng/L which was significantly higher than that in controls (102.60±12.69 ng/L). The mean value of ejection fraction were 62.23±3.46% and 63.80±4.34 in cases and controls respectively. Pro12Ala polymorphism was present in 2 out of 30 (6.67%) β-TM patients with osteoporosis. One patient had heterozygous 12Ala polymorphism and the other had homogygous 12Ala polymorphism. Both had normal body mass index, lipid profile, ejection fraction and elevated serum ferritin (4923 ng/l in heterozygous patient and 4886 ng/l in homogygous patient). Ejection fraction was 70% in heterozygous patient and 68% in homogygous patient. Only one male control (10%) has homozygous 12Ala polymorphism (Table 1).

Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cases (n=30)</th>
<th>Control (n=10)</th>
<th>x²</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>36.1±6.0</td>
<td>37.5±4.5</td>
<td>0.21</td>
<td>0.64</td>
</tr>
<tr>
<td>Sex</td>
<td>17 (56.7%)</td>
<td>7 (70.0%)</td>
<td>0.21</td>
<td>0.64</td>
</tr>
<tr>
<td>Body mass index</td>
<td>19.8±2.0</td>
<td>20.2±1.5</td>
<td>0.09</td>
<td>0.76</td>
</tr>
<tr>
<td>Ferritin (ng/l)</td>
<td>4886</td>
<td>4923</td>
<td>1.02</td>
<td>0.31</td>
</tr>
<tr>
<td>TRIGlycerides (mg/dl)</td>
<td>127</td>
<td>103</td>
<td>0.21</td>
<td>0.64</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>130</td>
<td>161</td>
<td>0.21</td>
<td>0.64</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>68</td>
<td>75</td>
<td>0.21</td>
<td>0.64</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>25</td>
<td>22</td>
<td>0.21</td>
<td>0.64</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>62.23±3.46</td>
<td>63.80±4.34</td>
<td>0.21</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Summary/Conclusions: This study suggests that Pro12Ala polymorphism may have a cardioprotective effect in Egyptian thalassemic patients since we find the highest value of ejection fraction among the two positive cases. Further studies on a larger population of patients are still needed to confirm this finding.

PB2193

THALASSEMIA MAJOR AND INTERMEDIA IN PATIENTS OLDER THAN THIRTY-FIVE YEARS - FROM A FATAL TO A CHRONIC DISEASE

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Background: During the past four decades beta thalassemia major (TM) and beta-thalassemia intermedia (TI) have transformed from a universally fatal disease to chronic condition. We aimed to characterize disease and patients' characteristics in patients above 35 years of age in an adult thalassemia center in Israel.

Methods: We conducted a retrospective analysis of 14 adult patients over the age of 35 years with TM (N=10) and TI (N=4) treated in a single center, specializing in the care of adult thalassemia patients. We used descriptive statistics to describe characteristics of disease and patients and the Mann-Whitney test to compare between patients with TI and patients with TM.

Results: Between 2006 and 2016, 14 adult patients older than 35 years with TM (n=10) and TI (n=4) were followed and treated in our center. Median patients' age was 37 (range, 35-51) years, with 66% males and 50% of Arab ethnicity. Most of the patients had at least high school education (85%), and 78% were employed. Thirteen patients (all TM patients and 3 out of the 4 TI patients) were treated regularly with blood transfusions. All patients received chelation treatment. Median hemoglobin (Hb) levels and mean corpuscular volume (MCV) levels were lower in patients with TI compared to TM (8.1 vs 10 g/dl, p=0.002 and 72.4 vs 84 fl, p=0.004, respectively). Median LDH levels and indirect bilirubin levels were higher in patients with TI compared to TM (603 vs 330 u/L, p=0.004 and 2.02 vs 1.1 mg/dl, p=0.06, respectively) indicating increased hemolysis. All patients underwent splenectomy and had secondary thrombocytosis. All but two patients were treated with at least two different chelation modalities, either as single agent, including subcutaneous (SC) or intravenous (IV) deferoxamine (DFO), deferriprone (DFP), or deferasirox (DFX), or as various combination therapy options. The median number of chelation treatment lines was 3. All patients treated with chelation suffered from at least one adverse event, necessitating temporary discontinuation and usually substitution of treatment. The median number of adverse events was 1.5 per patient. Nine patients (64.2%) had good compliance with current chelation therapy. Four patients with acute heart failure secondary to cardiac iron overload, and all four improved with intensified chelation treatment. Four TM patients (40%) were hypothyroid, half of them requiring thyroid hormone replacement therapy. All TM patients had hypogonadism. All females had amenorrhea and were treated with hormone replacement therapy, and none of them tried to conceive. Six of the seven male TM patients were treated with monthly testosterone injections, and three of them fathered children. All TM patients had osteoporosis, and three TI patients (75%) had metabolic bone disease. Figure 1 shows the relative rates of symptomatic cardiac iron overload and endocrine dysfunction in the cohort. Three patients (21.4%) had significant liver overload according to liver T2* MRI, necessitating chelation treatment intensification. None of the patients in our cohort underwent allogeneic hematopoietic stem cell transplantation and none developed secondary malignancy during follow-up.

Summary/Conclusions: Advances in the treatment of thalassemia patients have enabled the majority of these patients prolonged survival into adulthood. However, this has brought a new set of challenges for both patients and health-care. This study delineates the challenges faced while treating adult patients with TI and TM in the new era.
(group 1) and moderate to severe group (group 2). In addition to standard packed red cell transfusion, Spirulina therapy was given orally for 3 months, after which re-evaluation of these children was performed by repeating the same investigations.

**Results:** There was significant increase in LIC associated with significant changes in other MRI parameters in LIC >15 mg Fe/gr d.w.) and 3 had heart iron overload, of which one significant (T2* 1.9 msec) (Table 1).

**Results:**: There was significant increase in LIC associated with significant changes in other MRI parameters in moderate to severe group as compared to those of the mild group before treatment. The mean values of serum ferritin (SF) was statistically insignificantly higher among patients of mild group. There was no significant correlation between different MRI parameters and SF level. There was negative correlations between LIC and T2* and positive correlation between LIC and R2*.

**Background:**: Frequent transfusions required for β-thalassemia major patients cause iron overload. Without the appropriate chelation therapy, iron toxicity can cause significant heart, liver and endocrine morbidity. In this case series we estimated the safety and efficacy of iron chelation with the combination of deferasirox (DFX) and deferoxamine (DFO) in transfusion dependent thalassemia (TDT) patients attending the Thalassemia Unit in a tertiary hospital in Athens, Greece.

**Methods:**: 10 TDT patients were treated with a combination chelation therapy of DFX (30 ±10mg/kg/d) and DFO (44±12mg/kg/d for 2-6 days/wk in 12hr or 22hr infusion rates). Reasons for starting this combination treatment included: 1) treatment with one chelating agent did not succeed in decreasing heart and liver iron overload, 2) adverse events recorded with increased doses of one of the three different sites for the Premier Hb9210TM and of 100 normal samples and 217 patient samples for a variety of beta-thalassemia trait and haemoglobin (Hb) variants, including the molecular data of the beta-thalassemia mutations and Hb variants.

**Summary/Conclusions:**: All three apparatuses identified the common Hb variants and beta-thalassemia trait in carriers, homo-, hetero- and compound hemoglobinopathy with the expected sensitivity and specificity. The Premier Hb9210TM HPLC provide a reliable, high resolution HPLC of Trinity Biotech (Menarini) comparable separation and quantitation between the three different sites using the same sample and is suitable for the analysis of samples suspected of having haemoglobinopathy and the diagnosis of beta-thalassemia trait or Hb variants.

**Background:**: System® XE-5000 analyzer incorporates new research Red Blood Cell (RBC) parameters, derived from flow fluorescence cytometry technology, including%HYPO-He, which indicates the percentage of RBC with haemoglobin (Hb) content <17 pg, and%MicroR which indicates the percentage of RBC with mean cell volume <60 fl.

**Methods:**: The reference ranges of our Laboratory for the parameters%HYPO-He &%MicroR are 0.0 – 0.6% & 0.2 – 2.9%, respectively, and they are independent of gender and age (P=0.715, P=0.168 & P=0.073, P=0.843). There was no statistically significant difference between the different apparatuses identified the common Hb variants important to be diagnosed in multi-ethnic populations found in the U.K., the Netherlands and Northern Italy as well as elevated HbA2, as indicator for beta-thalassemia carriers.

**Methods:**: 1Clinical Genetics, Leiden University Medical Centre, Leiden, Netherlands, 2Special Haematology, Guy’s Hospital, London, United Kingdom, 3Laboratorio di Genetica Umana, Ospedale Galliera, Genoa, Italy
the total number of patients included was 31 (9 Thalassemia Major (TM), 1 Thalassemia Intermedia (TI), 21 other not sickle-hemoglobinopathies). The center follows 209 sickle patients, which leads to a ratio sickle/not sickle of 6.74 (Table 1). Ratio boy/girl is 1.21 for all group. Most of patients were born in Spain (90.32%), although 6.45% were born in Asia and one patient was born in Romania. Considering the parents, 32% were born in Europe, 29% from Asia, 25% from Africa, 12% from America. 92% of those patients born in Spain were detected in their first days of life due to universal screening detection implemented in Madrid since 2003. Median age at first diagnosis was 0.70 years (0-16.35). Median age at the end of inclusion was 9.39 years (range 1.90 to 35.44). 35% of them had molecular genotyping for diagnostic confirmation. The outcomes of 10 patients with Thal had HLA identical siblings. Quelation treatment was added to standard treatment to all the patients with Thal: 7 received deferasirox, 3 were treated with deferoxamine and 2 with deferiprone; 2 of the patients required double quelation. Two out of 10 patients with Thal underwent two years prior to registration was 24 PRBC units. The mean age at start of chelation was 10.0 yrs. Mean duration of chelation was 14 yrs. Majority (88%) had growth failure with mean height of 159.6 cm & mean weight of 51.5 kg respectively. Splenomegaly was present in 47% and hepatomegaly in 25% patients. Twenty-eight percent have undergone splenectomy at an average age of 12.6 yrs. The mean of highest ferritin levels was 6131 ng/ml and the mean ferritin at the time of registration was 2919 ng/ml. LFT were deranged in 25% of patients. Evidence of cardiac dysfunction (ECG/MUGA) was present in 22% of patients. Iron overload in liver and heart as measured with T2* MRI was present in 56% & 28% respectively (Figure 1).

Figure 1.

Summary/Conclusions: majority of patients registered in our clinic are living a healthy life. All of them were on iron chelation therapy and the dose was being adjusted as per the serum ferritin level. Amongst asymptomatic individuals with no evidence of cardiac or hepatic dysfunction, evaluation by T2* MRI picked up evidence of hepatic and cardiac iron overload. Therefore, its prudent to monitor patients with T2* MRI and accordingly escalate or de-escalate chelation therapy.
spleenectomy. None of these patients had sepsis or meningitis. Three Thal patients underwent progenitor stem cell transplantation and they remain on complete chimerism in the present момe. Patients lost to follow-up summed up 14; 3 emigrated to other countries, 2 continue the monitor of their diseases in other centers or in adults units and 7 for unknown reasons. There was one death (3.22%) for a cause unrelated to his illness.

Table 1.

Summary/Conclusions: Early diagnosis derived from universal neonatal screening for sickle cell disease allows an effective health education and prompt therapy to other hemoglobinopathies, and a correct and thorough follow-up of these patients.

PB2201
PREVALENCE AND CAUSES OF CLOTTING TIMES PROLONGATION IN PATIENTS WITH TRANSFUSION DEPENDENT BETA THALASSEMIA
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Background: Thalassemia is traditionally known to be a thrombophilic, rather than hemorrhagic, disorder. In spite of this, prolongation of clotting times are often reported. Understanding if there is a real risk of bleeding, and what this risk can be associated to, is crucial, especially in relation to the frequent referral to surgery (e.g. for splenectomy, cholecystectomy). Hepatopathy due to iron overload or HCV infection has been addressed as a main cause of this finding, even though disorders in the clotting profile are often reported also in patients with no alterations of hepatic function. The impairment of factors XI and XII often reported has been hypothesized to be secondary to intravascular haemolysis or multiple transfusions (Caocci et al, Acta Haematol 1978, Mcfadyen et al, Ann Hematol 2014), but no data are available to confirm this supposition.

Aims: To determine the prevalence of clotting disorders in a group of Transfusion dependent Thalassemia (TDT) patients and to assess the correlation with hepatopathy, degree of the hemolysis, transfusion frequency, erythroblastosis, iron chelation.

Methods: TDT patients followed at our center for whom clotting tests were available were included. From chart review data were collected regarding clotting times, demographics, disease history, comorbidities and concomitant medications, iron chelation therapies, iron overload (serum ferritin, LIC, cardiac T2*), liver function tests, hemolysis parameters, hemocromocitometric values. Patients on anticoagulation therapy were excluded.

Results: 100 TDT patients (female 55,35%) were enrolled in our study, mean age 26.02±13.38 years, 17 of them were pediatric. In 20/56 patients (35,71%) prolongation of clotting time was found: this included both prolonged INR (23,21%) and prolonged aPTT ratio (25%); 7 patients (12,5%) had both prolonged INR and aPTT. Subgroup with clotting disorder (group A) was compared to subgroup with clotting times within normal ranges (group B) using T-Test. No differences were found in terms of sex, age, genotypy, transfusion interval, hemolysis degree, comorbidities, HCV infection included, iron overload, liver function, erythroblastosis and platelets levels, nor in history of thrombotic complications. No patients had history of hemorrhagic disease. Pretransfusion Hb was lower in patients with prolonged clotting times (p=0.045); none of the patients in Group A was splenectomized (p=0.042).

Summary/Conclusions: In our population clotting disorders were not correlated with hepatic disease, nor hemolysis or transfusions. The mild correlation with lower Hb values and with the lacking splenectomy could be consistent with the known effect of low Hb on lab procedures for clotting tests. In relation to this observation in patients with altered coagulation tests the repetition of clotting test after blood transfusion could be advisable to overcome the low Hb effect.
PB2203

ANTITHROMBOTIC EFFECTS OF PEPTIDE PGPL IN EXPERIMENTAL THROMBUS FORMATION

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Background: Previously, it was established that proline- and glycine-containing peptides have fibrinolytic, anticoagulant activity, inhibit platelet aggregation and thrombin activity in vitro and in vivo. Besides, it is known that short peptides of this family also have antithrombotic effects. Aims: To study the influence of Pro-Gly-Pro-Leu (PGPL) and amino-acid leucine on fibrinolytic and anticoagulant blood activity, platelet aggregation and to estimate their possibility to reduce the formation of experimental blood clots. Methods: Experiments were carried out on white rats (200-250 g) according to the ethical principles of the Helsinki Declaration. Peptide PGPL (1 mg/kg), leucine (0.33 mg/kg - equivalent to its content in PGPL) and saline (control rats) were intranasal entered to rats within 3 days. 1 hour after the last drugs administration we induced the formation of thrombus in n Jugularis (Wessler model). The degree of thrombus formation was estimated on thrombus weight. Fibrinolytic activity and activity of tissue-plasminogen activator (t-PA) of blood plasma were measured by fibrin plate method. Anticoagulant activity (APTT-test) and ADP-induced platelet aggregation were detected by standard methods. Results: Our experiments demonstrated that preliminary intranasal administration of PGPL (before formation of thrombus) leads to increase of APTT, fibrinolytic and t-PA activity on 18%, 62%, 35% accordingly from control rats. Besides, we observed the decrease of platelet aggregation. Also we indicated the reduction of thrombus weight in PGPL-treated rats on 68.5% comparatively with control rats. The thrombus weight after leucine treatment decreased on 30% compared with control rats. But administration of leucine did not change of haemostasis system parameters. Summary/Conclusions: Thus administration of PGPL enhanced anticoagulant, fibrinolytic and antiplatelet activity in rats blood plasma. PGPL pretreatment lead to prevention of experimental venous thrombus formation. Therefore, PGPL may be used as a perspective anticoagulant and fibrinolytic agent with direct antithrombotic effect.

PB2204

TREATMENT AND OUTCOME OF THROMBOTIC MICROANGIOPATHY IN MALAYSIA

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Background: Thrombotic Thrombocytopenic Purpura (TTP) is a potentially lethal disease that there is still no promising cure in this era. The ADAMTS-13 deficiency or defect in the disease has enabled clinician to recognize another entity which is Thrombotic Microangiopathy (TMA). This entity includes TTP, HUS, Pregnancy TMA, SLE related TMA, Transplant TMA. Results: Only 54 (24.15%) patients were diagnosed as TTP based on ADAMTS-13 activity ≤10%. Treatments were evaluated by using complete case details from Ampang Hospital via the electronic hospital information system (EHIS) and external records of NPUH. ADAMTS-13 activity was ≤10% (11.4 cycles) as compared to those with ADAMTS-13 activity >10% (7.7 cycles). The odds of relapse is 2.9 times higher given the ADAMTS-13 activity ≤10% to ADAMTS-13 activity >10% (log-rank, p<0.0001). Besides, thromboembolism, either provoked or spontaneous. The odds of relapse is 2.9 times higher given the ADAMTS-13 activity ≤10% to ADAMTS-13 activity >10% (log-rank, p=0.0299). Therefore treatments were as a result of plasma exchange or plasma infusion. Summary/Conclusions: This study illustrated that the standard treatment like plasma exchange and immunosuppressant therapy are only effective in genuine TTP whereas those masquerading TTP (TMA) would be more challenging to be tackled in terms of improving the outcome. The task to investigate other types of TMA prospectively will be highly desirable in the future.

PB2205

ANTIPHOSPHOLIPID ANTIBODY PROFILE AND ORGAN INVOLVEMENT IN CRITICALLY ILL PATIENTS WITH AUTOIMMUNE DISEASES

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1Universidad de La Sabana, 2Hospital Universitario de la Samaritana, 3Fundación Santa Fe De Bogotá, Bogotá, 4Universida de La Sabana, Bogotá, Colombia

Background: Antiphospholipid antibodies (APA) are a group of proteins directed against the phospholipids of cell membranes, such as cardiolipins or phospholipid binding proteins. APA presence provokes microvascular, arterial or venous thrombotic events indicating somehow the relationship between the immune system, the hemostatic system, and the inflammatory response. It has been suggested that their presence in a critically ill patient is related to thrombotic manifestations, organ dysfunction, and death. Aims: The aim of this study was to evaluate the prevalence of antiphospholipid antibodies in critically ill patients with autoimmune diseases and the rate of organ involvement. Methods: Retrospective and descriptive study of patients admitted to the intensive care unit of Hospital Universidad de la Samaritana between 2008 and 2016, in Bogotá, Colombia. Results: A total of 79 patients were found to have systemic lupus erythematosus (SLE), antiphospholipid syndrome and vasculitis. 17 patients (22%) were positive for antiphospholipid antibodies. Of these, 76% were women and mean age was 38 years (18-63 years). APA profiles showed positivity with this distribution: one positive antibody, n=9 patients (53%) (lupus anticoagulant antibody being the most common), two positive antibodies in n=4 patients (23%) and three positive antibodies in n=4 patients. Anemia (100%), monocytosis (64%), thrombocytopenia (40%) and prolonged INR (17%) were found in 88% of patients on admission to the ICU. In descending order, other organ involvement was found to be: pulmonary and renal dysfunction (70%), shock (53%), central nervous system involvement (41%), cardiovascular (23%), and gastrointestinal (22%). 82% of this cohort had positive anti-nuclear antibodies (ANA) and 23% anti-platelet antibodies (ANCA). 100% of patients had elevated C-reactive protein (CRP), and APACHE II score average was 11 points (Table 1).

Table 1.

Phospholipid binding proteins activity distribution.

Summary/Conclusions: Hematologic, renal and pulmonary involvement are the most commonly compromised in patients with antiphospholipid antibodies positive. In this cohort of critically ill patients with autoimmune diseases in the ICU. Based on these results, a prospective study is proposed in order to evaluate the presence of APA and their impact on mortality and multi-organ dysfunction in these patients.
Background: Antiphospholipid antibodies (APLs) have been implicated in vascular (arterial, venous or both) thrombosis. Diabetes Mellitus (DM), as a disease entity has been associated with hyper-coagulable and pro-thrombotic states, with studies showing an increased procoagulant state and thrombotic events especially in poorly controlled Type 2 Diabetes Mellitus (T2DM).

Aims: The aim of the study is to assess the APLS and HbA1C levels and evaluate the correlation between APLS levels and HbA1C in T2DM patients with diabetic vascular complications.

Methods: This was a cross-sectional study of subjects with T2DM attending the diabetic clinic of University of Nigeria Teaching Hospital. A total of two hundred and ten (210) subjects were recruited for this study. There were grouped into two (2) groups: T2DM - uncomplicated T2DM and health control. Each had 70 subjects matched for sex and age. Lupus anticoagulant (LA) was assayed using DRVVT (technocnome GmbH Austria) IgGβ2GPI-ACA was assayed using ELISA test kit (Genway Bio-tech San Diego USA), HbA1C was assayed using D10TM haemoglobin analyzer. Ethical clearance was obtained from the ethical committee of UNTH.

Results: The prevalence of LA was 7.1%, 4.3% and 4.3% for complicated T2DM, uncomplicated and healthy control subjects respectively, while the prevalence of IgG-B2GPI ACA was 4.3% in all groups. The mean HbA1C were 8.2(1.5), 8.0 (1.7), 5.6 (0.38) for complicated, uncomplicated T2DM and healthy controls respectively. ANOVA showed a significant difference in mean HbA1C among complicated uncomplicated T2DM and healthy controls. Post hoc analysis showed this difference was between complicated T2DM and healthy controls (p<0.001, 95% CI-3.0 to -2.1) and in uncomplicated T2DM and healthy controls subjects (p<0.001, 95% CL-2.8 to -2.0) there was a significantly lower HbA1C in HbA1C and IgGβ2GPI-ACA for complicated T2DM (r=0.316), P=0.008 and uncomplicated T2DM (r=0.316), P=0.001

Summary/Conclusions: The study did not find any causal or other association between T2DM and the occurrence of APLS positivity, however, APLS may be simply an aggravating factor for vascular complications especially in poor controlled T2DM.

PB2207

VWF THRX89AALA GENETIC VARIANTS CORRELATE WITH DISEASE PHENOTYPE IN EGYPTIAN PATIENTS WITH ACUTE CORONARY SYNDROME

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Background: von Willbrand factor antigen level (vWF: Ag) was shown to contribute to the risk of cardiovascular disease. vWF Thr789Aala single nucleotide polymorphism is thought to affect factor level and function.

Aims: This study aimed to investigate the APLS genetic variants at that position on the risk of acute coronary syndrome (ACS).

Methods: The study included 112 patients of ACS, 31 with unstable angina (UA) and 81 with myocardial infarction (MI) as well as 118 healthy controls. vWF: Ag level was measured by ELISA. The gene analysis was carried out by polymerase chain reaction using restriction fragment length polymorphism (RFPL-PCR) principles.

Results: vWF: Ag levels were significantly higher in MI (111.6±24.77 IU/dl) and UA (110.27±23.44 IU/ml) patients compared to healthy controls (71.13±13.72 IU/dl), p<0.001 for both groups. The majority of patients with UA (80.6%) were Ala789 homozygous, 33.1% were heterozygous and 19.5% were Thr789 homozygous. Regarding the MI group, Ala789 geno-type was present in 48.1% of patients and Thr789 homozygous was present in 17.3% of patients. The genotype frequency in the control group was as follow; 47.4% were Ala789 homozygous, 33.1% were heterozygous and 19.5% were Thr789 homozygous. The genotype distribution was significantly different among the 3 groups, p<0.001, and between the groups with UA and MI, p<0.001. Ala789 homozygous genotype was an independent risk factor for UA while the Thr789Aala genotype was shown as an independent risk factor of MI.

Summary/Conclusions: In conclusion, vWF Thr789Aala single nucleotide polymorphism is independent risk factor for UA and has significant impact on the type of myocardial ischemia. It should be incorporated in a risk assessment model to identify individual patient risk and guide the management plan.

PB2208

THE INFLUENCE OF FIBRINOGENASE ISOLATED FROM THE ANTARCTIC SCALLOP ON BLOOD COAGULATION

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Background: At the present time, cardiovascular diseases such as acute myocardial infarction, ischemic heart diseases, and stroke are the most important causes of the human mortality around the world. Thrombosis is probably the most common symptom among cardiovascular diseases. Thrombolytic agents have been extensively used in the therapeutic treatment of thrombosis. But most of them have some serious shortcomings, including limited efficacy, short plasma half-life, long therapeutic dose or allergic response. Considering the global burden, the search continues for a safe and cheap thrombolytic agent to treat cardiovascular diseases. To date, many investigators have been trying to improve the safety and efficacy of thrombolytic therapy. Fibrinogenolytic agents and fibrinogenases because of their role in solving of blood clots as well as prevention their formation have attracted special medical and scientific attention. Enzymes that affect hemostasis have been isolated from different sources. In recent years, special attention is paid to the hydrobionts from the Antarctic region which are poorly explored and potentially can be a valuable source of new bioactive compounds, in particular enzymes.

Aims: The main goal of current research was to test the effect of fibrinogenase from marine hydrobiont the Antarctic scallop Adamussium colbecki on platelet aggregation and blood coagulation.

Methods: Fibrinogenase from the crude tissue extract of A. colbecki was isolated using sequential chromatography on Sepharose following affinity chromatography on Blue-Sepharose and size exclusion chromatography on Superdex 75-PC. Platelet aggregation was determined by AT-02 aggregometer (Meditech, RF). The platelet count was adapted to 2.5x10^11 platelets/L with platelet-poor plasma. Then, fibrinogenase (12.5 μg/mL or 6.25 μg/mL) was added 2 min before the addition of the platelet aggregation inducer (5x10^-6 M ADP). The changes in light transmittance were continuously monitored during 8 min, and the percentage of aggregation, in the presence of the samples, was calculated comparing the transmittance against the controls. Activated partial thromboplastin time (APTT) and prothrombin time (PT) were coagulation tests for monitoring, due to less variability in drug effect for a given dose, however it’s recommended monitoring the drug for Rivaroxaban Apixaban and Edoxaban use anti-Xa chromogenic studies and for Dagibatran Hemoclot thrombin inhibitor and Ecarin clotting time (DTI test).

Aims: Determine the effectiveness of laboratory tests to monitor patients treated with direct oral anticoagulants.

Methods: We conducted a retrospective study with 227 patients who received direct oral anticoagulants (DOACs) between January 2015 and December 2016. One hundred eighteen patients (52%) receive Rivaroxaban, fifty patients (22%) receive Dabigatran and fifty nine patients receive Apixaban (28%). We analyzed the variables that increases the bleeding risk such as age, prothrombin time (PT) and activated partial thromboplastin time (aPTT), therapeutic range of the drug, and measurement of serum creatinine.

Results: We found 10% of toxicity with Dabigatran, 7% with Rivaroxaban and a 3% with Apixaban. Thirty-five patients (15%) developed bleeding of which 1% of patients died a minor bleeding of 4% of patients had a major bleeding. We also found that 6% of patients with Dabigatran, 2.5% with Rivaroxaban and 1.5% with Apixaban developed thrombotic episodes. Twenty percent of patient didn’t have therapeutic range of the drug. For each DOACs is shown in Table 1. When we analyzed the patients who had hemorrhage we found that all patients with Dabigatran prolonged aPTT and PT in 80%, for other DOACs is shown in Table 1. A retrospective case-matched analysis was performed comparing 35 patients who developed bleeding with an equal number of patients who did, case and control groups were matched according to age, weight and measurement of serum creatinine. We didn’t find significant difference.

Summary/Conclusions: In summary, in patients with dabigatran and who suffered bleeding, we found a significant prolongation of aTTP and PT, demonstrating the importance of laboratory tests prior to the administration of these agents and in emergency situations, for these reason should be include PT.
and aPTT, therapeutic level of the drug and creatinine measurement, within the emergency and control laboratory tests in patients that receive DOACs.

Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Apixaban</th>
<th>Rivaroxaban</th>
<th>Dabigatran</th>
</tr>
</thead>
<tbody>
<tr>
<td>percentage</td>
<td>25.9%</td>
<td>31.8%</td>
<td>22%</td>
</tr>
<tr>
<td>toxicity</td>
<td>3.3%</td>
<td>6.9%</td>
<td>10%</td>
</tr>
<tr>
<td>thrombophilic episodes</td>
<td>1.8%</td>
<td>2.5%</td>
<td>6%</td>
</tr>
<tr>
<td>percentage out of therapeutic range</td>
<td>8.4%</td>
<td>25.4%</td>
<td>23%</td>
</tr>
<tr>
<td>prolonged aPTT</td>
<td>8.4%</td>
<td>2.6%</td>
<td>80%</td>
</tr>
<tr>
<td>prolonged PT</td>
<td>16.9%</td>
<td>21%</td>
<td>4%</td>
</tr>
<tr>
<td>Bleeding</td>
<td>(33) 15.4%</td>
<td>(36) 20%</td>
<td>7.8%</td>
</tr>
<tr>
<td>percentage</td>
<td>34.3%</td>
<td>20.6%</td>
<td>7.8%</td>
</tr>
<tr>
<td>prolonged aPTT</td>
<td>8.3%</td>
<td>22.9%</td>
<td>100%</td>
</tr>
<tr>
<td>prolonged PT</td>
<td>25%</td>
<td>35.3%</td>
<td>80%</td>
</tr>
<tr>
<td>mean therapeutic range</td>
<td>177</td>
<td>142</td>
<td>154</td>
</tr>
<tr>
<td>mean serum creatinine</td>
<td>Imol/L</td>
<td>Imol/L</td>
<td>Imol/L</td>
</tr>
<tr>
<td>mean age</td>
<td>81</td>
<td>80</td>
<td>70</td>
</tr>
</tbody>
</table>

1Haematology & Immunology, 2Internal medicine, University Of Nigeria Teaching Hospital, Enugu, 3Internal medicine, Abia State Teaching Hospital, Umuahia, 4Internal medicine, Nnamdi Azikiwe Teaching Hospital Nnewi, Awka, 5Internal medicine, Federal Medical Centre, Abakiliki, 6Internal medicine, Amaku Specialist hospital, Awka, Nigeria

Background: Thromboembolic and hypercoagulable diseases are common life-threatening but treatable problems in hospital practice. The most effective and economical approach to decreasing the burden of VTE is to prevent the development of DVT and PE in patients especially in acutely ill hospitalized medical patients. Health care providers in Nigeria may have significant gaps in their anticoagulation knowledge that could affect their decision to prescribe anticoagulation therapy as there are no national guidelines on the use of anticoagulation in Nigeria.

Aims: The purpose of this present study was to examine the knowledge and attitude of medical doctors on anticoagulation in tertiary hospitals in Nigeria.

Methods: The present study is a multicentre survey of the use of anticoagulants among clinicians in South East Nigeria. A pretested questionnaire was administered to clinicians in six tertiary hospitals in the south-east of Nigeria. The following institutions participated in the survey: University of Nigeria Teaching Hospital Enugu, Federal Medical Centre, Abakiliki, Federal Medical Centre Umuahia, Abia State Teaching Hospital, Aba, Amaku Specialist Hospital, Awka and Nnamdi Azikiwe Teaching Hospital, Nnewi. The Likert scale which is in grades from one to five: 1 strongly disagree, 2 disagree, 3 neutral, 4 agree, 5 strongly disagree was used. To determine the agreement degree three levels of agreement (high medium and low).

Results: There were 528 respondents. 378 of them were males (71.6%) and 150 were females (28.4%). 31.1% of the respondents, were junior residents and the consultants represented only 20.6% of the respondents. Most of the respondents, 189 (35.8%) had less than 5 years clinical experience while the least of the respondents (8.7%) had between 16-20 years clinical experience. We observed that most respondents irrespective of their job grades didn’t know about Fondaparinux and the DOAC (except those in the specialist - registrar job grades) as the overall p(0.000),<0.05 and was significant. We also observed that responses were divided on malignancy as an indication for anticoagulation. The overall p=0.002, <0.05 and was significant. The p value for other indications for anticoagulation >0.05 and was not significant. The majority knew of protrombin time and p value was 0.03, less than alpha value of 0.05 and was significant. On the contrary, Majority does not know about anti-Xa assay, p-value=0.02, <0.05 was also significant. Their affirmative response on the mode of action as one of the differences showed a p=0.000, <0.05, was significant. On the contrary, the non-affirmative response to drug and food interaction, p=0.03, was also significant. Based on results of the statement analysis, the variables were ranked according to the value of their mean. All except one variable had p-values of <0.05. The statement “Do you think antiocoagulation therapy/prophylaxis is clinically important” had the highest mean of 4.60 and had a high degree of agreement. The statement “Should hospital inpatient with >3 days admission routinely receive anticoagulation?” had the lowest mean of 2.27 with a p-value of 0.015 had a low degree of agreement.

Summary/Conclusions: There is a need to upscale knowledge attitude and practice of the use anticoagulants agents especially the NOACs through well-articulated CME educational activities. A limitation of this study is the relatively small number of study participants and some subspecialties that were not reflected in this survey.

PB2214

INTHERLEUKIN -10 GENE POLYMORPHISMS AND THE RISK OF UNPROVOKED DVT IN EGYPTIAN PATIENTS

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Background: Thrombosis is often multifactorial, caused by both genetic and acquired risk factors. The inflammatory process is linked to pathogenesis of venous thrombosis. Venous thrombosis is considered to be mediated by an imbalance in pro-inflammation as compared with anti-inflammation factors. One of the important anti-inflammatory cytokine is interleukin-10 (IL-10) with important immunoregulatory functions. Primarily, IL-10 counterbalances the potentially harmful effects of tumor necrosis factor α (TNFα) and other pro-inflammatory mediator such as IL-1, IL-6, and IL-8 from monocytes/macrophages. Three important single nucleotide polymorphisms were described to include IL-10 expression, including: 1082 A/G, 819 C/T, and 592 C/A. Studying the association between genetic polymorphisms of anti-inflammatory cytokines such as IL-10, and venous thrombosis may suggest using of polymorphisms as a predictive genetic marker of future VTE.

Aims: The objective of this study was to evaluate a possible association between IL-10 -1082A/G, and -592C/A polymorphisms with DVT.

Methods: The study was conducted on 115 patients with symptomatic DVT proved by venous duplex ultrasound; divided into two cohorts: group A included...
60 patients with unprovoked DVT, and group B included 55 patients with provoked DVT. Gene mutations for IL-10 -1082AG, and -592CA were performed using PCR-restriction fragment length polymorphism assay. We studied the association between IL-10 gene polymorphisms and occurrence of either provoked or non-provoked DVT. We also investigated the link between these polymorphisms and the recurrence of DVT and family history of DVT.

Results: Among group A (provoked DVT) as compared to group B (unprovoked DVT), AG genotype was detected in 14 patients (63.6%) versus 8 patients (36.4%) in group A and B respectively (P value = 0.037); AG genotype was detected in 30 patients (66%) compared to 17 patients (36.2%) in group A and B respectively (P value = 0.007). However, there is no correlation was found between IL101082 mutant genotypes distribution and VTE recurrence (P value = 0.738 and 1 respectively) or positive family history of VTE (P value = 0.101 and 0.714 respectively), compared to wild genotype. IL10592AC gene analysis showed that mutant genotypes (AG and AC) distribution showed no statistically significant difference (P value = 0.43 and 0.687 for GG and AG genotypes respectively) compared to wild genotypes distribution, also there is no correlation was found between IL10592AC mutant genotypes distributions and VTE recurrence (P value = 1 and 0.284 for GG and AG genotypes respectively) compared to wild genotype (AA).

Summary/Conclusions: IL101082AG gene polymorphism is associated with risk of unprovoked DVT, however it is not associated with either risk of recurrence or positive family history.

PB2215

CATASTROPHIC ANTI-PHOSPHOLIPID SYNDROME TRIGGERED BY SEPSIS: A PROSPECTIVE CASE STUDY HIGHLIGHTING BIOLOGICAL CONCEPTS AND MANAGEMENT STRATEGIES IN THIS COMPLEX AND LIFE THREATENING DISEASE M. Huá

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Background: Catastrophic antiphospholipid syndrome (CAPS) is a rare and life threatening event characterized by widespread intravascular thrombosis and multi-organ failure, often triggered by a preceding infectious event.

Aims: To study the effect of tripeptides Pro-Arg-Gly and Gly-Arg-Pro, containing arginine in healthy rats and rats with experimental MS.

Methods: A prospective case study illustrating two separate atypical APC presentations and the management strategies employed. 1st episode (2015): 54F with long standing 27 years of triple positive APS, pro-thrombotic history with recurrent thrombosis despite optimal anticoagulation. Her pro-thrombotic equivalents were hyperhomocysteinemia as a respiratory trigger and severe depression.

Results: Among study sample, 134 patients had strokes and only 18 had TIA. The recurrence of stroke/TIA was observed in 13.2% of patients. The majority of patients (94.7%) had radiological evidence of thrombotic event. One fourth of patients had past thrombotic events while 12.5% had family history of thrombosis. Out of haematological correlates screened Lupus anticoagulant was the most common haematological correlate (n = 16) and dysfibrinogenemia(11) had the next high prevalence. One patient was diagnosed with Essential thrombocythemia and one with SLE. None of the patients were positive for screening tests done for sickle cell disease and PNH.

Summary/Conclusions: The haematological correlates were identified in 19% of our study sample. Among stroke profile only presence of past thrombotic event and history was statistically significant associated with haematological disorders (P = 0.04). Therefore hemodilatory disorders appear to be an important factor in etiological work up of stroke patients particularly in patients with past thrombotic events.

PB2217

ANTIPLATELET AND FIBRINOLYTIC EFFECTS OF ARGinine-CONTAINING PEPTIDES IN HEALTHY RATS AND RATS WITH METABOLIC SYNDROME Y. Song1,* M. Grigorjeva1, T. Obergan1

1M.V.Lomonosow Moscow State University, Moscow, Russian Federation

Background: Currently, the number of diabetes, hypercholesterolemia, metabolic syndrome (MS) patients has increased sharply in the world. MS is metabolic disorders with increase of cholesterol and glucose levels, dyslipidemia, endothelial dysfunction. This is accompanied by an increase in blood clotting, including platelet aggregation strengthening and reducing the activity of the plasminogen activator. Thus, the MS may predispose to venous thrombosis. It is known that, regulatory oligopeptides involved in the conservation normal functional activity of coagulation, anticoagulation, insular systems of the organism, fat metabolism. It is also known that some amino acids, particularly arginine, improve rheological properties of blood and reduce platelet aggregation. It is known that, regulatory oligopeptides involved in the conservation normal functional activity of coagulation, anticoagulation, insular systems of the organism, fat metabolism. It is also known that some amino acids, particularly arginine, improve rheological properties of blood and reduce platelet aggregation.

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Summary/Conclusions: The haematological correlates were identified in 19% of our study sample. Among stroke profile only presence of past thrombotic event and history was statistically significant associated with haematological disorders (P = 0.04). Therefore hemodilatory disorders appear to be an important factor in etiological work up of stroke patients particularly in patients with past thrombotic events.

PB2216

HAEMATOLOGICAL CORRELATES OF ISCHEMIC STROKE AND TRANSIENT ISCHEMIC ATTACK : LESSONS LEARNED H. Gunasekara1, I. Pathirage2

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Background: Haematological abnormalities are known to cause ischemic Stroke or Transient Ischemic Attack (TIA). The identification of haematological correlates plays an important role in management and secondary prevention.

Aims: The objective of this study was to describe haematological correlates of stroke and their association between stroke profile. The haematological correlates screened were Lupus Anticoagulant, Dysfibrinogenaemia, Paroxysmal nocturnal haemoglobinuria (PNH), Sickle cell disease, Systemic Lupus Erythematosis (SLE) and Myeloproliferative Neoplasms (MPN).

Methods: A cross sectional descriptive study was conducted in a sample of 152 stroke patients referred to haematology department of National Hospital of Sri Lanka for thrombophilia screening. Following tests were performed to assess each hematological correlates (Table 1).

Table 1.

<table>
<thead>
<tr>
<th>Hematological correlate</th>
<th>Tests performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lupus anticoagulant</td>
<td>Anticardiolipin VLA test and Kaolin clotting time</td>
</tr>
<tr>
<td>Sickle cell Disease</td>
<td>Full blood count (FBC), blood picture and thick kite high Fibrinogen level</td>
</tr>
<tr>
<td>Paroxysmal nocturnal haemoglobinuria</td>
<td>5’s, Blood picture, full test and Erythrocytosis</td>
</tr>
<tr>
<td>Myeloproliferative diseases</td>
<td>Renal function tests, urine and creatinine renal level and base line examination</td>
</tr>
</tbody>
</table>

Diagnosis: Followed by anticardiolipin antibody and anti-murphy antibody.

Results: Among sample study, 134 patients had strokes and only 18 had TIA. The recurrence of stroke/TIA was observed in 13.2% of patients. The majority of patients (94.7%) had radiological evidence of thrombotic event. One fourth of patients had past thrombotic events while 12.5% had family history of thrombosis. Out of haematological correlates screened Lupus anticoagulant was the most common haematological correlate (n = 16) and dysfibrinogenemia(n=11) had the next high prevalence. One patient was diagnosed with Essential thrombocythemia and one with SLE. None of the patients were positive for screening tests done for sickle cell disease and PNH.

Summary/Conclusions: The haematological correlates were identified in 19% of our study sample. Among stroke profile only presence of past thrombotic event and history was statistically significant associated with haematological disorders (P = 0.04). Therefore hemodilatory disorders appear to be an important factor in etiological work up of stroke patients particularly in patients with past thrombotic events.
PB2218

THE PRINCIPAL COMPONENT ANALYSIS USING CALIBRATED AUTOMATED THROMBOGRAM PARAMETERS AS A POTENTIAL QUALITY CONTROL FOR MEASURING PROCOAGULANT ACTIVITIES OF IMMUNOGLOBULINS

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Background: The calibrated automated thrombogram (CAT) is a method to monitor the generation of thrombin. It can be described by four variables: lag time, peak thrombin, time to peak, and velocity index. Currently, due to thromboembolic event related risks of immunoglobulins, the CAT is widely used to quantify the thrombogenic potential associated with immunoglobulin manufacturing processes and products. However, there is currently no officially approved method for such assessments and even this result is highly variable in inter-laboratories comparison. In this study, to obtain a summary score, we applied the principal component analysis (PCA) for these four outcomes measured from CAT method. The PCA is a statistical procedure concerned with elucidating the covariance structure of a set of variables. In particular it allows us to identify the principal directions in which the data varies.

Aims: In this study, our interest is to apply PCA method in order to find appropriate dose related with CAT variables and to reduce variation of procoagulant values in Immunoglobulin products.

Methods: The CAT are measured in a 96 well plate fluorometer equipped with a 390/460 filter set and a dispenser. Usually experiments are carried out in triplicate. During the measurement, a dedicated software program, Thrombinoscope compares the readings from the trigger wells and the calibrator wells, calculates thrombin concentration and displays the thrombin concentration in time. Outcomes from CAT were analyzed in the principal component analysis (PCA) which is a statistical procedure that allows us to summarize high dimensional data with a smaller number of representative variables that collectively explain most of the variability. Statistical analyses were performed with R 2.5.

Results: Four variables measured from CAT have different distribution and too large variations. For example, the mean(sd) of each variable (lag time, peak thrombin, time to peak, and velocity index) are 24.66(8.01), 80.16(94.52), 31.28(9.78), 19.08(28.86), respectively. Therefore, to remedy such high variability among variables and to find a score, PCA method is applied. Then the dose values calculated based on the PCA scores have mean 0.393 and a much smaller variation (sd=0.583) (Table 1).

Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lag Time (min)</td>
<td>24.66 (8.01)</td>
</tr>
<tr>
<td>Peak Thrombin (s)</td>
<td>80.16 (94.52)</td>
</tr>
<tr>
<td>Time to Peak (s)</td>
<td>31.28 (9.78)</td>
</tr>
<tr>
<td>Velocity Index</td>
<td>19.08 (28.86)</td>
</tr>
</tbody>
</table>

Summary/Conclusions: The PCA value showed a good agreement with four CAT outcomes and less variation. The PCA method could be used to monitor the process of immunoglobulin manufacturing.

PB2219

PRIMARY THROMBOPHILIA IN MÉXICO XII: MISCARRIAGES ARE MORE FREQUENT IN PERSONS WITH THE STICKY PLATELET SYNDROME

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Background: The sticky platelet syndrome (SPS) is an inherited condition which leads into arterial and venous thrombosis. There is scant information about the association between the SPS and obstetric complications.

Aims: To assess the relationship of the SPS and fetal loss in a single institution.

Methods: The obstetric history of all the consecutive female patients prospectively studied along a 324 month period, in a single institution with a history of thrombosis and a clinical marker of primary thrombophilia was reviewed.

Results: Between 1989 and 2016, 268 consecutive patients with a clinical marker of primary thrombophilia and a history of arterial or venous thrombosis were studied; of these, 108 were female patients. Within this subset of thrombophilic female persons, 77 (71%) had been pregnant at some moment. Twenty eight of these 77 patients (37%) had had a spontaneous abortion and 24 out of these (86%) were found to have the SPS. On the other hand, in a subset of 73 female patients with the SPS who had been pregnant, 32% had miscarriages. These figures are significantly higher than the prevalence of abortions in the general population of pregnant women, with p<0.013 (chi square=7.47; p=0.0063). Accordingly, the relative risk of having a miscarriage is 2.66 times higher in female patients with the SPS than in the general population (p=0.0014) (Figure 1).

Figure 1.

Summary/Conclusions: In México, female patients with the SPS experience significantly more spontaneous abortions than the general population. Since the treatment of the SPS is simple and effective and could in turn prevent adverse obstetric outcomes, its investigation in women studied because obstetric complications may be useful and deserves further research.

PB2220

CROSS-SECTIONAL ANALYSIS OF VENOUS THROMBOEMBOLISM IN YOUNG INDIAN MALES; NEW INSIGHTS INTO AN OLD PROBLEM

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Background: Venous thromboembolism (VTE) comprising of deep vein thrombosis (DVT) and pulmonary embolism (PE) is one of the major cardiovascular causes of death along with MI and stroke. Though earlier works suggested that DVT is rarer in Asian population, recent studies have revealed that this might not be so. Most of the studies conducted in Asia in general and India specifically has been on hospitalized patients with minimal representation of young healthy individuals.

Aims: We aimed at studying the disease variables of VTE in young healthy males of Indian origin and compare the same with other Indian studies as well as the global statistics.

Methods: Hospital records of 176 Color Doppler Flow Index (CDFI) and/or Contrast Enhanced Computed Tomography (CECT) proven VTE patients being followed up in a tertiary care hospital was analyzed retrospectively to document cause (provoked/unprovoked), venous systems involved, thrombophilia profile, duration of anti-coagulation and recurrence.

Results: Among the study population, 49.8% had a provoked VTE. 90.9% subjects had DVT, mostly of the lower limb. 15.3% had PE with DVT, 2.8% had PE alone and 6.2% had splanchnic vein thrombosis including portal vein thrombosis. In the subjects who had undergone thrombophilia profile, 41.9% had Protein C, 58.1% Protein S and 25.9% Antithrombin III deficiency. Lupus anti-coagulant screen was positive for 13% of the screened subjects. The average duration of anti-coagulation was 16 months with majority (98.2%) patients on Vitamin K antagonist. The recurrence rate in our study population was found to be 11.4% (Table 1).

Summary/Conclusions: Young Indian males have different disease variables...
for VTE as compared to western population. The exact pathophysiology of such differences needs to be studied further to formulate strategies for effective screening and prevention.

Table 1.

**PB2221**

A PRELIMINARY STUDY ON THE EFFECTS OF AMPHIBIAN CRUDE SKIN SECRETIONS ON SOME PARAMETERS OF HEMOSTATIC SYSTEM

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**Background:** A lot of bio-chemical compounds from secretion of the amphibian skin glands with various biological activities have been isolated and characterized. Several recent studies indicate that amphibian skin secretions can be a source of molecules affecting the platelet activity. We are interested to look for other bioactive components of the amphibian skin which exhibit ability to influence on diverse parameters of hemostatic system.

**Aims:** We performed a preliminary study of the some effects of amphibian crude skin secretions on hemostasis.

**Methods:** Adult specimens (both sexes) of Bombina bombina, Bombina variegata, Bufo bufo, and Bufoes viridis were collected from outdoors in Kyiv region of Ukraine. The crude skin secretions were collected by washing with ultrapure water and centrifuged to remove debris. The supernatants were prepared water solution of lyophilized skin secretions. Protein concentration was determined by Bradford method with BSA as a standard. Rabbit platelet-rich plasma (PRP, 2x10^4 cells/µL) and platelet-poor plasma were obtained following standard protocols. Platelet fraction (PF) was purified by gel-filtration on Sephadex G 50 column. Platelet aggregation was measured by aggregometer AT-02 (Medtech, Russia). Coagulation parameters (prothrombin time (PT), thrombin time (TT), as well as activated partial thromboplastin time (APTT)) were measured by coagulometer (Rayto, RT-2201C) using corresponding commercial kits (Renam, Russia).

**Results:** The lyophilized B. bufo skin secretions in dose-dependent manner induced platelet aggregation in both PRP and purified PF; its final concentration of 50 mg of total protein/mL caused the same effect as 5x10^{-6}M ADP. These results indicated that skin components acted directly on platelets, maybe through their surface receptors. The lyophilized skin secretions of B. variegata and B. bufo also activated platelet aggregation but their effects were lower than B. bufo skin secretions. The skin secretions from all studied amphibian did not influence on PT and TT except B. viridis which prolonged TT by 40%. The values of APTT were significantly enhanced in 3.4 and 2.3 times under the influence of crude skin secretions (final concentration of 0.2 mg total protein/mL, plasma) of B. bombina and B. variegata, respectively.

**Summary/Conclusions:** The obtained results indicate the prospects of the B. variegata, respectively.

**PB2222**

**THE TREATMENT OF HEREDITARY TROMBOPHILIA DURING PREGNANCY**

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**Background:** Thrombophilias are genetic conditions that increase the risk of thromboembolic disease. The use of anticoagulant therapy during pregnancy is challenging because of the potential for both fetal and maternal complications. The most common complication is venous thromboembolism.

**Aims:** This study is conducted in order to assess the importance of treatment during pregnancy for women with hereditary thrombophilia, the risks of not treating the disease or treating incorrectly.

**Methods:** This study includes a total of 207 women, from which 83% were treated with low molecular weight heparin and Aspirin during pregnancy regardless if it was their first pregnancy or not and the rest 17% remained untreated during pregnancy. The success of the treatment is based on the completion of the pregnancy and the good health of the fetus.

**Results:** A total of 207 women were included into the study, 172 were treated with low molecular weight heparin and Aspirin while 35 were treated with just Aspirin. Out of 172 patients in the low molecular weight heparin group 155 managed to give birth which accounts for a 90% success rate with a reported case of fetal growth restriction and 2 cases of abruptio placenta which were successfully managed. The remaining 17 women which represent the 10% of the treated patients were unsuccessful in completing their pregnancy with 14 women presenting pregnancy loss on the first trimester and 2 having late fetal death, only one case of preeclampsia was recorded. Out of the 35 women who did not receive treatment with low molecular weight heparin and only with Aspirin, 21 managed to complete their pregnancies representing the 60% out of which 2 cases presented with Abruption and 4 cases with fetal growth restriction, out of the 14 women who represent the 40% who were unsuccessful in completing their pregnancies 7 cases were recorded during the first trimester while 3 more had late fetal loss and 4 cases of preeclampsia.

**Summary/Conclusions:** Women treated for thrombophilia had a lower percentage of fetal loss than their no treatment group counterparts. There is an urgent need for appropriate guidelines for these patients in our medical center.
Aims: To ascertain D-dimer diagnostic accuracy for upper extremity DVT.

Methods: A retrospective audit was undertaken to determine the aetiology and clinical presentation on patients who UDVT at presentation. Patients with a formal malignancy confirmed before the diagnosis was excluded. A D dimer (DD) with a cut off cut off levels validated for lower limb DVT was performed.

Results: A total of 18 patients was identified in the period of 2012 to 2016. All the cases investigations included Doppler US or CT/MRI and in 30% of the patients the thrombosis was confirmed via contrast venography as a reference standard test. The gender predominance was male in this group the symptomatology were related to physical efforts in a 60% (Paget-Shroetter Syndrome) whereas in female serie the predominant was thrombiocpic defects (factor V Leiden). The average age was 33 years (ranging from 21 to 68 years) and 2 elderly patients a new diagnosis of cancer was confirmed (thyroid and lung) (odds ratio, 3.24; 95% CI, 1.13-9.38). The 85% of the patients had an unprovoked event; four patients have a diagnosis of catherer related thrombosis and four cases intravenous drug abuse (factor V Leiden). Two patients had a diagnosis of SLE. We had four cases of positive DD screening (both were marginally elevated, P <0.01) . The risk of re-thrombosis was non significant but in the subanalysis of relapsing thrombotic event populations the risk of relapse increased proportionally in relation of thrombiocpic defect and high BMI. A trend towards a higher rate of recurrent thrombosis (was observed among patients with BMI>25 (42.6%) compared to those with a BMI <25 (33%). This difference reached statistical significance in women with BMI>25, who had recurrent event in 51.7% of the cases vs those with BMI<25 (29.7%) (p <0.05 CI 0.03; 0.41).

Summary/Conclusions: In the unprovoke serie the relation of DD was positive in less tan 30% of the cases and none statistically significant (p<0.01). In the case of subclavian vein occlusion this is result in limited clot burden (which explain the correspondence with negative DD value). The risk of re-thrombosis is associated with thrombiocpic defect and high BMI exclusively. The DD adjust ed odds ratio for the studied population was 1.48 (95% CI 0.38-6.26). The DD cut off adapted of the specific population). A prospective studies of DD in suspected UDVT need to be adressed.

PB2225

THE INFLUENCE OF HEPARINOID FROM THE PEONY ROOTS ON THE THROMBUS DISSOLUTION

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2Medical department, Kuban State Medical University, Krasnodar, Russia

Background: Many plants have an effect on the blood clotting system. It is known that there are heparin-like substances in some types of peony roots (Paeonia lactiflora, Paeonia suffruticosa). It proved that there is an anticoagulant activity in extracts from such roots.

Aims: The intention is to show the inhibitory effect of the extract of Paeonia lactiflora roots (EA) on processes fibrin and thrombus formation.

Methods: We used the standard coagulographic methods for determining anticoagulant activity by APTT test, antiplatelet, total fibrinolytic activity (TFA), fibrindepolymerizing activity (FDPA). Experiments were carried out in accordance with ethical principles and documents recommended by the Declaration of Helsinki of the humane treatment of animals. We used an animal model with provoked thrombosis in experimental rats by administration of subthreshold doses of tissue thromboplastin at a dose of 0.6 -0.7 ml per 200 g body weight in rats. After 30 min after injection of thromboplastin, we injected intraperitoneal-ly 0.1 mL of 1% of extract of EP and after 30 minutes we determined parameters of hemostasis in the blood plasma.

Results: It was shown that after administration of the indicated doses thromboplastin occurs hypercoagulability in blood plasma of animals (APTT decreased by 23% SFA - 15%, FDPA -12%; increased platelet aggregation by 18% compared to control animals not receiving thromboplastin). Normalization of blood clotting is installed in the experimental rats after application EP (recov-er of platelet aggregation to 98%, APTT- to 100%, up to 95% SFA- FDPA and up to 67% compared with control). The high degree of FDPA indicates the ability of EP to obstruct the process of the formation of fibrins and thrombosis. Heparin components in EP interact with fibrin monomers which do not partici-pate in their conversion to fibrin polymer. As a result, stable fibrin polymer or thrombus is not formed.

Summary/Conclusions: Consequently, the extract of Paeonia lactiflora roots containing heparinoid contributes to the restoration of coagulation properties in blood of animals in prothrombotic condition and prevents thrombosis. In the initial stages of fibrin formation, it causes the thrombus dissolution.

PB2226

LOW MOLECULAR WEIGHT HEPARIN AND HIGH MOLECULAR WEIGHT HEPARIN: COMBINATION WITH ADRENORECEPTOR ANTAGONISTS AND PREVENTION OF THROMBUS FORMATION

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2Medical Department of Moscow State University, Moscow, Russian Federation

Background: Rethrombosis and thromboembolism are the most common side effects of thrombolytic therapy. One of the possible causes of thrombosis is the entering of thromboplastin in the blood stream. Marker of thromboplastin is an intrinsic membrane glycoprotein 5'-nucleotide (5'NT) that is present as an enzyme in a wide variety of cells. Recently it was shown that compensatory reaction of haemostasis system by using different fibrinolytic drugs was connected with the stimulation of the sympathetic nervous system. Besides, it is known that α-adrenoreceptors play an important role in the initiation of fibrinolytic and antiplatelet effects. The prevention of thrombosis complication is very important field of pathophysiology and medical practices. Therefore, we studied effects of different α–adrenoreceptor antagonists and the influence of these substances combinations with various anticoagulant and fibrinolytic agents.

Aims: The study of the influence of low molecular weight heparin (LMWH, 4.4 kD) and high molecular weight heparin (HMWH) and their combinations with different α–adrenoreceptor antagonists (AA) on experimental thrombosis preven-tion, and the influence of LMWH and HMWH on α–adrenoreceptor blockers in the process of thrombus dissolution.

Methods: Experiments were carry out on 50 white laboratory rats weighing 200-230 g according to the ethical principles of the Helsinki Declaration. Anti-coagulant and antithrombotic effects of LMWH or HMWH were studied in two rat models of thrombosis – thrombosis in v. jugularis (Wessler) and thrombosis in arterio-venous shunt (direct registration of blood pressure). The α–AA digy- droergotoxin (DET – 1mg/kg), α1–AA prazosin (PZ – 2mg/kg), LMWH or HMWH (40 USP/kg were injected in v.jugularis. Saline was administrated in control rat groups. The thrombus were formed 15 or 180 min after substances injected. The degree of thrombus formation (TF) was detected in ball (Wessler model) and by time of TF (arterio-venous shunt model). In blood plasma the activity of 5'NT was detected. The experiments were process in accordance with ethical principles and documents recommended by the Declaration of Helsinki.

Results: The increase of anticoagulant and antithrombotic effects of LMWH or HMWH by pretreatment of DET or PZ were shown in both animal models of venous thrombosis. The degree of TF by Wessler model may be estimated as 3.7 (saline), 1.2 (LMWH), 1.8 (HMWH), 0.9-1.1 (DET+ LMWH or PZ+LMWH) and 1.1-1.3 (DET + HMWH). Besides it was shown that the TF was accompanied with significant hypercoagulation of blood: 5'NT activity was increased in 2 time comparatively with normal level. LMWH or HMWH combi-nations with DET or PZ administration led to normalization of 5'NT level in blood plasma. In arterio-venous shunt model it has been shown that the time of TF was 2 min (saline), that was accompanied with the decrease of blood pressure (on 40-50 mmHg). In this case the time of TF was prolonged in 4 time (LMWH) or 2 time (HMWH) comparatively with saline group 15 min after injection; in 4-5 time (DET+ LMWH or PZ+LMWH) or 3-3.5 time (DET+HMWH or PZ+HMWH ) comparatively with saline group 180 min after injection.

Summary/Conclusions: Thus we confirmed that LMWH (as one, as in com-bination with α–adrenoreceptor antagonists) has definite advantages over HMWH. Besides our results show that α–adrenoreceptor antagonists signifi-cantly improve antithrombotic effect of anticoagulents agents (LMWH and HMWH). Therefore the combination of LMWH with selective and nonsel ective α–adrenoreceptor antagonists may be effective used for prevention of venous thrombosis development and thromboembolia.

PB2227

THE POLICY AND PRACTICE OF ANTICOAGULATION THERAPY AMONG CLINICIANS IN SOUTHERN NIGERIA.

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1Haematology & Immunology, 2Innate disease, University Of Nigeria Teaching Hospital, Enugu, 3Internal medicine, Abia state Teaching Hospital, Umuahia, 4Internal medicine, Nnamdi Azikiwe Teaching Hospital Nnewi, Awka, 5Internal medicine, Federal Medical center , Abakiliki, 6Internal medicine, Amaku Specialist hospital, Awka, Nigeria

Background: In the absence of anticoagulation therapy, the risk of Venous thromboembolism: deep-vein thrombosis (DVT) and pulmonary embolism (PE) is medically ill patients comparable to that in moderate-risk surgical patients. Previous studies have revealed grossly inadequate knowledge and a dismal practice of anticoagulation among healthcare workers in some resource poor countries. Prophylactic anticoagulation is under-prescribed in Nigeria, South Africa, as well as in many other countries in Africa. The aim of the study was to evaluate the practice of anticoagulant therapy. It will also document the frequency of drug-induced complications resulting from the use of anticoagulants and presence of an anticoagulation policy in the hospitals surveyed.

Methods: This is a multicentre cohort survey of the practice of anticoagulant therapy on blood coagulation during many years. Cases were identified by means of validated questionnaires among cardiovascular patients. The questionnaire was designed to assess their practices anticoagulation. The questionnaire was administered consecutively on clinicians in the participating centers. The following institutions participated in the survey: University of Nigeria Teaching Hospital, Enugu, Federal Medical Centre Abakiliki, Federal Medical Centre Umuahia, Abia State Teaching Hospital, Aba and Amaku Specialist hospital Awka. Statistical package for Social Science (SPSS) software, version 18 (SPSS Inc., Chicago, IL) was used for analysis.
Results: A total of 528 clinicians were involved in the survey. There were more males 378 (71.6%) than females, 150 (28.4%). The clinicians who practiced for less than 5 years are in the majority 189 (35.8%) and those with 15–20 years of practice 46(8.7%) are in the minority. Only 52 of the respondents (9.8%) claimed their institutions had an anticoagulation policy while 274 (51.9%) of them said there was no such policy and 168 (31.2%) do not know of any policy. Unfractionated heparin was the most frequently used (96.8%) and fondaparinux was the most infrequently used (42%). Most of the prescriptions were done by younger clinicians who are the highest in number. The consultants prescribed heparin and warfarin most, with the newer anticoagulants taking the rear position. Only 193 (36.6%) of the respondents routinely prescribed anticoagulation therapy when indicated. 412(78%) of respondents believe the risk of anticoagulation outweighs the benefits while 439 (83.1%) identified cost as an important variable in prescribing anticoagulation agent. Anti-coagulation prophylaxis was the most frequently used for patients immobilized or bedridden (94.1%); malignancy and atrial fibrillation were the most infrequent reasons for using anticoagulation agents (50.6%). A total of 63 respondents (11.9%) were not satisfied and 219 (41.5%) were not very satisfied with the laboratory monitoring tool available in their institutions. Bleeding is the most common complication of anticoagulation while the least encountered complications are skin and jaw necrosis among the respondents 492(93.2%), 1(0.2%) respectively.

Summary/Conclusions: This survey has shown the lack of anticoagulation policies among the centers that participated. Our survey has also shown deficiencies in the areas of practice of anticoagulation among the clinicians in the Southeast of Nigeria. These gaps can be remedied by continuous medical educations in the areas of practice of anticoagulation among the clinicians in the hospitals of the region. Following implementation of control and continuous monitoring measures while establishing proper procedures such as transfusion guidelines, administration of blood and blood products and Maximum Surgical Ordering Practice, Mafraq blood bank, supported by the Transfusion and Tissue & Quality & Patient Safety Committees, achieved a great success in reducing C:T ratio beyond the acceptable target and ranged between 2.5 to 3.2 highlighting the need for blood in hospitals continues to exceed the volume collected by the transfusion services. The gross over-ordering of blood, in excess of actual and anticipated needs leads to substantial costs and a burden to the transfusion services. In addition, over-ordering leads to non-availability of cross-matched units for other patients who might be in urgent need of transfusion.

Background: The need for blood in hospitals continues to exceed the volume collected by the transfusion services. The gross over-ordering of blood, in excess of actual and anticipated needs leads to substantial costs and a burden to the transfusion services. In addition, over-ordering leads to non-availability of cross-matched units for other patients who might be in urgent need of transfusion.

Aims: We are aiming to reduce the Cross-match-to-transfusion ratio (C:T ratio) & improve blood utilization in Mafraq Hospital.

Methods: In 2011 the ordering practice at Mafraq Hospital, a designated Trauma Centre, had been evaluated. Data collected retrospectively over a one year period and a C:T ratio adopted by the American Association of Blood Bank was calculated for all various subspecialties including Surgery, Internal Medicine, Pediatrics and Obstetrics and Gynecology. All procedures related to hospital transfusion practice have been retrieved and re-evaluated to address gaps. Policy of maximum surgical blood ordering (MSBO) was implemented based upon both results of audits and by discussion and agreement between medical teams. Focused training and education has been followed to increase the awareness of the health care workers. Plus monitoring of C:T ratio on monthly basis, blood bank team had arranged meetings with the departments that were over-ordering cross-matches to explain that group & save test is a safe, effective and financially beneficial strategy. Communicating with the physicians had been the most challenging aspect of implementing the policy changes. Regular audits had been conducted to measure the compliance and effectiveness of the blood management practice.

Results: Compared to the international guidelines, C:T ratios in 2010 was beyond the acceptable target and ranged between 2.5 to 3.2 highlighting the over-ordered cross-matched blood in certain sub-specialties. This practice of ordering was probably because of the fear that blood will not be available, if needed. Following implementation of control and continuous monitoring measures while establishing proper procedures such as transfusion guidelines, administration of blood and blood products and Maximum Surgical Ordering Practice, Mafraq blood bank, supported by the Transfusion and Tissue & Quality & Patient Safety Committees, achieved a great success in reducing C:T ratio <2 all through 2016 Figure 1. The reduction of C:T ratio had improved blood inventory control and reduced the workload of the blood bank staff. Because fewer units of cross-matched PRBC are being ordered, the blood bank has been able to decrease the number of expired units &reducing money loss Figure 1. The savings in technologist time is particularly significant since the blood bank is most of the time at a minimal staffing level.

PBZ228

UMBILICAL CORD BLOOD PLASMA INFUSION PROMOTES BLOOD CELL RECOVERY IN INPATIENTS WITH ACUTE LEUKEMIA UNDERGOING CHEMOTHERAPY

Background: Umbilical cord blood plasma (UCBP) is separated from umbilical cord blood. UCBP contains a variety of hematopoietic growth factors which can stimulate hematopoiesis.

Aims: The aim of this work is to explore the influence of UCBP infusion on blood cell recovery in patients with acute leukemia undergoing chemotherapy.

Methods: Patients with the diagnosis of acute leukemia were included in this study and they were randomly distributed to experimental group and control group. Patients in experimental group received infusion of 100ml UCBP with the same ABO and Rh blood type every day after chemotherapy for five days and patients in control group received placebo for the same time. Blood routine tests were tested every day until WBC >4.0×10⁹/L and PLT >20×10⁹/L.

Results: 25 patients were included in the study of which 23 were brought into statistics. 13 patients were in experimental group and 10 in control group. There were no difference in age, gender and dose intensity of chemotherapy between the two groups (P>0.05). The average recovery time of the blood neutrophil granulocyte >0.5×10⁹/L in experimental group and control group were respectively (6.52±3.26) days versus (12.92±4.75) days (P<0.05) and that of PLT >20×10⁹/L was respectively (9.24±3.88) days versus (13.15±5.76) days (P<0.05). No UCBP transfusion-related side effects were found.

Summary/Conclusions: UCBP administration is safe as treatment for cytopenia and could promote blood cell recovery in patients with acute leukemia undergoing chemotherapy.

PBZ229

TOWARD BETTER BLOOD TRANSFUSION PRACTICE: A SUCCESSFUL RED BLOOD CELL UTILIZATION TOOLS IN A TERTIARY CARE HOSPITAL

Background: The need for blood in hospitals continues to exceed the volume collected by the transfusion services. The gross over-ordering of blood, in excess of actual and anticipated needs leads to substantial costs and a burden to the transfusion services. In addition, over-ordering leads to non-availability of cross-matched units for other patients who might be in urgent need of transfusion.

Aims: We are aiming to reduce the Cross-match-to-transfusion ratio (C:T ratio) & improve blood utilization in Mafrq Hospital.

Methods: In 2011 the ordering practice at Mafraq Hospital, a designated Trauma Centre, had been evaluated. Data collected retrospectively over one year period and a C:T ratio was adopted by the American Association of Blood Bank was calculated for all various subspecialties including Surgery, Internal Medicine, Pediatrics and Obstetrics and Gynecology. All procedures related to hospital transfusion practice have been retrieved and re-evaluated to address gaps. Policy of maximum surgical blood ordering (MSBO) was implemented based upon both results of audits and by discussion and agreement between medical teams. Focused training and education has been followed to increase the awareness of the health care workers. Plus monitoring of C:T ratio on monthly basis, blood bank team had arranged meetings with the departments that were over-ordering cross-matches to explain that group & save test is a safe, effective and financially beneficial strategy. Communicating with the physicians had been the most challenging aspect of implementing the policy changes. Regular audits had been conducted to measure the compliance and effectiveness of the blood management practice.

Results: Compared to the international guidelines, C:T ratios in 2010 was beyond the acceptable target and ranged between 2.5 to 3.2 highlighting the over-ordered cross-matched blood in certain sub-specialties. This practice of ordering was probably because of the fear that blood will not be available, if needed. Following implementation of control and continuous monitoring measures while establishing proper procedures such as transfusion guidelines, administration of blood and blood products and Maximum Surgical Ordering Practice, Mafraq blood bank, supported by the Transfusion and Tissue & Quality & Patient Safety Committees, achieved a great success in reducing C:T ratio <2 all through 2016 Figure 1. The reduction of C:T ratio had improved blood inventory control and reduced the workload of the blood bank staff. Because fewer units of cross-matched PRBC are being ordered, the blood bank has been able to decrease the number of expired units &reducing money loss Figure 1. The savings in technologist time is particularly significant since the blood bank is most of the time at a minimal staffing level.
PB2230
SAFETY AND EFFICACY OF A PROTHROMBIN COMPLEX CONCENTRATE IN VKA REVERSAL AND OFF-LABEL INDICATIONS
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Background: Prothrombin complex concentrates (PCC) are highly purified mixtures of plasma coagulation factors that contains vitamin K dependent and anticoagulopathy factors, they are approved for urgent reversal of vitamin K antagonists (VKA). Massive bleeding-associated coagulopathy guidelines include PCC in their management, although as an off-label indication.

Aims: The aim of the present work is to evaluate safety and efficacy of PCC in a case series of VKA reversal and refractory coagulopathy associated with major bleeding.

Methods: Retrospective review of cases treated with a four-factor PCC between January 2010 to January 2016 in two tertiary Universities Hospitals. As safety endpoints we evaluated infusion reactions and incidence of thromboembolic events by self reported registry. The efficacy endpoints were studied in two separate cohorts: 1) INR correction for VKA reversal and 2) coagulopathy correction and early mortality (24 hours) in major bleeding coagulopathy.

Results: 328 patients were included (47.25% male), median age 78 years (range 19-102), PCC was used in the following cases: 1) 66.67% in VKA reversal indications (181 patients due to hemorrhage and 33 prior to emergent surgery), mean dose of PCC 1333.51 IU; 2) 30.54% in refractory coagulopathy in major bleeding (30 patients due to refractory bleeding protocol activation, 43 patients in hemorrhage coagulopathy and 25 patients in bleeding not related with any of previous reasons) a mean dose of PCC 1681.63 IU was used. Safety endpoint: Two infusion reactions were reported potentially related to PCC use, they were not specified neither as anaphylaxis nor as pulmonary edema, and 8 thrombotic episodes were observed (2.4%); 5 pulmonary embolism, 2 deep venous thrombosis and 1 portal thrombosis. 73% of the events appear in the group of VKA reversal. Efficacy endpoint: VKA reversal in bleeding patients was effective in 97% of them, 76.5% with complete reversal of INR value (INR<1.5), 34.25% of patients required red blood cell (RBC) transfusion, with a mean of 1.32 RBC. Prior to in vivo procedure VKA reversal was effective in 83% of patients, all procedures taking place with no bleeding complication, 36.3% of patients needed RBC with a mean of 1.12 units. 24 hours mortality in refractory coagulopathy associated to major bleeding was 31.6%, having a worse outcome (40% rate of death) those who suffer a massive bleeding coagulopathy, all death related with absence of bleeding control. A global INR correction happen in 76.7% of patients, complete correction in 40.7%. 63.26% received previous to PCC fresh frozen plasma. Invasive hemostatic procedures were required in 20% of the whole series.

Summary/Conclusions: A four-factor prothrombin complex was safe and effective as adjuvant treatment in refractory coagulopathy due to major bleeding as well as for the emergent reversal of VKA.

PB2231
TRACEABILITY OF RED BLOOD CELLS IN A HOSPITAL TRANSFUSION LABORATORY
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Background: According to European legislation (2002/98/EC, 2005/61/EC) as a requirement of hemovigilance system traceability (confirmation of final destination of blood components in hospitals) information should be kept for 30 years, improving the quality and safety of the transfusion process. Various methods are available from simple paper-based procedures to full electronic blood tracking systems. The ideal goal is to trace the final fate of 100% of the red blood cell (RBC) units, from donor to recipient and vice versa.

Aims: To check the ability to trace each individual unit from donor to recipient or disposal in our hospital.

Methods: To ensure compliance, the minimum traceability data set for retention is a mix of 1) Wards’ paper files (file of transfusions and/or patient records: 14/2 wards respectively), 2) HTL electronic records and paper records. The transfusion practitioner is responsible for the collection and maintenance of traceability data.

Results: During the year 2016, the number of RBC units transfused in our hospital was 2128. The traceability status of the transfused units is shown in the Table 1.

Table 1.

<table>
<thead>
<tr>
<th>Traceability of transfused units</th>
<th>Description</th>
<th>N</th>
<th>n total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confirmed</td>
<td>Full match of data</td>
<td>2067</td>
<td>1218</td>
</tr>
<tr>
<td>Presumed</td>
<td>Wrong number of units in the ward</td>
<td>25</td>
<td>2128</td>
</tr>
<tr>
<td>Presumed</td>
<td>No number of the units in the ward</td>
<td>0</td>
<td>2128</td>
</tr>
<tr>
<td>Unknown</td>
<td>No data in the ward*</td>
<td>29</td>
<td>2128</td>
</tr>
</tbody>
</table>

* Patients’ data has been archived for long-term retention.

Summary/Conclusions: Although we are satisfied that the results represent a reasonably accurate working model of the current situation, the trail of a unit is less reliable after blood has left the HTL. 1. Patients’ notes to provide traceability are not totally reliable. It is apparent that the ward staff plays a key-role part in the chain and this highlights the need for them to receive training to emphasize the importance of their contribution to hospital compliance. 2. The indications are that the essential requirements on traceability are not fully met by the current laboratory computer system. A configuration is needed to produce a report which lists components which have been assigned for use but do not have an entry for return to stock or final fate. Ongoing problems will be referred to the Hospital Transfusion Committee. 3. For the longer term ultimately only effective IT system in both wards and HTL can ensure total traceability and we recommend the inclusion of electronic tracking system in the National Blood Donor Registry Programme (EMA).
ed, after matching to refractory patients the frequency was 0.002%. Before matching to refractory patients, the frequency of NHFR was (0.03%) (Table 1).

Table 1.

<table>
<thead>
<tr>
<th>Status</th>
<th>Patients</th>
<th>Transfusions</th>
<th>Refractory without matching</th>
<th>% of all transfusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refractory before matching</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Non-refractory before matching</td>
<td>21</td>
<td>39</td>
<td>19</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Summary/Conclusions: The frequency of NHFR in groups with refractoriness with individual matching is significantly lower (10 fold) compared to groups with refractoriness before the matching (P<0.01)*.

PB2233
RARE DONORS AND MALARIA
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Background: Migratory flows of sub-saharan (SSA) persons throughout the world are expected to continuously increase. A significant proportion of SSA citizens are affected by Sickle Cell Disease (SCD), condition requiring repeated blood transfusions. Many centuries of malaria pressure have induced in SSA natives, homogeneous selection of peculiar haematologic characteristics, such as the absence of high frequency red cell antigens (defining a rare blood) that cannot be found in donors of European descent so that many SCD transfused patients experience the fearful occurrence of red cell alloimmunization. For these reasons haematologists are expecting to access to Rare Blood Banks in order to assure a full match between donor and recipient’s blood, that may be obtained from donors sharing the same ethnicity. Unfortunately SSA donor recruitment is counteracted by the widespread diffusion of infections contracted before migration: one of these is malaria. In SSA malaria may occur subclinically and is characterized by a slow antibody clearance. This peculiar condition, the so-called semi-immunity, has been induced by a strong genetic pressure, and is a kind of co-evolutionary process characterized by the co-existence and persistence of small entity of Plasmodium genome with relative antibodies. Molecular techniques are unreliable to detect a small number of Plasmodia, which may otherwise be sufficient to induce a transfusion transmitted malaria (TTM). The serologic assessment, despite the low specificity, remains the most sensitive and reliable method to detect the semi-immune status in blood donors (1).

Aims: The aim of this study was to assess the prevalence of malaria immunity in a cohort of healthy SSA citizens.

Methods: Since 2010 in our Department of Haematology and Transfusion Medicine we recruited 184 SSA citizens, in good health, who agreed to underwent clinical and laboratory investigations to become a blood donor. All of them were born in SSA Africa and lived there for at least the first 5 years of life. 70% of subjects didn’t recognize any previous malaria fever. The last travel/stay in Africa was 2-10 years (median 3 yrs), and 48% of returning people had received prophylaxis. Malaria serology was determined by a commercial enzyme immunoassay kit (Malaria EIA Ab, BioRad).

Results: Overall 75% of persons were positive for malaria antibodies. Serologic positivity was found in 75% of persons no more exposed in 5 recent years and even in 83% (19/23) persons settled in Italy since 10-20 years. Serologic positivity was present in 100% of people from Benin, 85% from Burkina Faso, 78% from Ivory Coast and Cameroon, 63% from Senegal. We followed antibody concentration in 50 persons (136 assays), and we observed a slightly negative trend that, in most cases, was followed by a prolonged phase of low antibody levels. 4/50 became negative after three years. 4/50 became negative after three years.

Summary/Conclusions: The identification of malaria antibodies is essential in SSA native donors and, by far, irreparable in order to avoid the risk of TTM. Until pathogen inactivation techniques will become available, we have a very low expectation to introduce SSA blood in Blood Bank inventories. Haematologists have to adapt some years for the forthcoming SSA second generation that will allow to fully match the entire SSDC patient community.

REFERENCE

PB2234
EFFICACY AND INFLUENCE OF IRON CHELATION THERAPY ON RED BLOOD CELL TRANSFUSIONS
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1Hematology, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, 2Hematology, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, 3Hematology, Baia-Mare County Hospital, 4Hematology, "Iuliu Hatieganu" University of Medicine and Pharmacy, Baia-Mare, Romania

Background: Chelation therapy is recommended for transfused patients that have an elevated serum ferritin level (over 1000 microg/l), evidence of iron overload or received over 20 units of red blood cell transfusions (RBCT). Deferasirox showedefficacy and safety in maintaining or reducing body iron (assessed by liveriron concentration or serum ferritin).Iron chelation therapy was associated with hematopoiesis improvement in transfusion-dependent patients and interruption of Deferasirox treatment of transfusions dependent myelodysplastic patients produced loss of erythroid response.

Aims: Aim of the study: to assess the results of Deferasirox efficacy, side effects and to study if the number of RBCT decreased after starting Deferasirox.

Methods: We have done a retrospective, transversal study including all the adult politransfused patients treated with Deferasirox in three counties Hematology Departments of Nord-West Romanian hospitals. Criteria of Deferasirox treatment: over 20 RBCT, serum ferriteine level over 1000 microg/l.

We created a data collection sheet including: demographics, information on patients’ disease, serum ferriteine level at start of and during treatment, Deferasirox dose, data about dose modification, adverse effects of Deferasirox and their management, reasons for treatment discontinuating, evaluation of comorbidities that could increase serum ferriteine level, number of RBCT before and after starting the treatment

Results: We included 40 politransfused patients treated with Deferasirox, age average 63. The diagnosis included mielodysplastic syndromes (most of patients), thalassemia, other anemias. Myelodysplastic patients were treated with low dose chemotherapy, epigenetic treatment, RBCT and other transfusion-dependent patients were transfused. The baseline value of ferritine was between 1075 - 6187 microg/l. Deferasirox dose: 20-30 mg/kg. There was a significant reduction in serum ferritine from baseline for all the patients. Ferritine median at start, 3631 microg/l decreases at 1537 microg/l after 6 months of treatment and at 894 microg/l after 12 months of treatment. There were 8 patients with ferritine decreased levels of ferritine, but during infectious episodes the ferritine increases for a short period of time. Digestive adverse events appeared in three cases (two cases of diarrhea and one case of digestive hemorrhagic episode). In all these cases the treatment was temporarily discontinuied. In three cases, treatment was stopped because low ferritin level (under 500 microg/l). RBCT were administered before (median 2.43 units/month) and after starting Deferasirox (mean 1.39 units/month), the difference is statistically significant (Student Test, t(39)=6.98, p<0.001). After starting Deferasirox treatment mean number of RBCT decreased, mean of differences (95% CI) was 1.04. We analyzed the group of 23 patients treated with Deferasirox less than 12 months, and the patients treated more than 12 months, 15 patients. In both groups the difference of RBCT means (before and after the start of the treatment) were statistically significant (for the patients treated less than 12 months: Student Test, t(23)=8.12, p<0.001 and for the patient treated more than 12 months: Student test, t(15)=3.03, p<0.008).

Summary/Conclusions: Analyzing our group of 40 patients, Deferasirox proves to be effective and safe. Adverse effects that determined a temporary stop of the treatment were mild/mid-term short time digestive reactions. The number of red blood cell transfusion significantly decreased after starting Deferasirox treatment.

PB2235
LIBERAL VS RESTRICTIVE COMPARATIVE TRANSFUSIONAL STUDY IN ONCOLOGICAL POPULATION
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Background: Allogeneic transfusion therapy is perhaps one of the most widely used treatments without good evidence support; despite many years of application in clinical practice. This, coupled with blood shortages, the impossibility to adequately cover the needs for patients that are at high risk, the lack of evidence that transfusion may increase consumption or decrease tissue oxygen debt and the existence of an association with an increase in morbidity and mortality have favoured that we join efforts towards its optimal use.

Aims: Optimal use in our adult oncological population and evidence that restrictive transfusion (TR, Hb 7-9 g/dl) is not greater or lowero the liberal transfusion (TL, Hb 8-10 g/dl), keeping hemoglobin in safe levels for the patient.

Methods: A research was performed from October 1st, 2015 through December 31st, 2016. We analyzed the proportion of patients receiving packed red cells (CH) and the number of units transfused as well as post-transfusion control in order to describe the outcome of the CH versus TL strategies in the cancer population under the study.

Results: See Table 1.

Summary/Conclusions: The results obtained in our series of 311 cancer
patients indicate that the restrictive strategy has been equally effective and probably superior to the liberal one maintaining Hb at a safe level in each patient, as well as quality of life and comfort in a subgroup with advanced and terminal cancer.

Table 1.

<table>
<thead>
<tr>
<th>Transfusion Therapy</th>
<th>Patients (N)</th>
<th>Hb Pre (g/dL)</th>
<th>Hb Post (g/dL)</th>
<th>yield CRI (g/visit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT</td>
<td>192</td>
<td>8.1</td>
<td>9.3</td>
<td>1.0</td>
</tr>
<tr>
<td>LT</td>
<td>57</td>
<td>9.7</td>
<td>9.4</td>
<td>1.0</td>
</tr>
<tr>
<td>PWC</td>
<td>23</td>
<td>8.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TPF</td>
<td>111</td>
<td>8.0</td>
<td>9.2</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Hb Pre: Pre-transfusion haemoglobin; Hb Post: Post-transfusion haemoglobin; PWC: Patients without post transfusion Hblevel; TPF: Total Patients Transfused; X- half haemoglobin.

PB2236

HIGH RISK OF HBV INFECTION IN VACCINATED POLYTRANSFUSED CHILDREN

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Background: Children receiving chemotherapy for neoplastic diseases are still susceptible to Hepatitis B virus (HBV) infection despite the national HBV vaccination program coverage for all infants since 1992. The aim of this study was to analyze immunity against HBV and occurrence of HBV breakthrough infections in polytransfused children who had been vaccinated during infancy.

Methods: The study included 89 children with hematological disorders and malignancies, who were categorized into group A (37 receiving chemotherapy (M:F 20:17; mean age: 7.7±4.0) and group B): 52 polytransfused children (M:F 31:21; mean age: 7.6±3.2). A matched healthy control group (n=162) was also included. All patients and controls had received their primary vaccination against HBV in infancy. Quantitative anti-HBs were tested for patients and controls. Patients’ sera were tested for HBsAg, anti-HBc, and HBV-DNA (nested PCR for serum pools & DNA only).

Results: Levels of anti-HBs between 10-100 IU/L and ≥100 IU/L were found among 13.5% and 21.6% [group (A)] 44.2% and 11.5% [group (B)] and 32.1% and 10.5% of controls respectively. A significant difference in HBsAb levels between patients receiving chemotherapy (group (A)) and both groups B patients (p<0.008) and controls (p=0.032). However, no difference was found between polytransfused children (group (B)) and controls.

HBsAg was positive in 21 (67.7%) children under chemotherapy [group (A)] compared to 10 (32.2%) polytransfused children [group (B)] (p<0.0005). Overall, 49 patients (55%) were HBV-DNA positive; 44 c-region positive, 7 s-region positive. 2 positive for both c and s-regions and one positive for c and x-regions. Of those, only 21 patients (42.8%) were also positive for HBsAg; while 28 (47.2%) had occult HBV infection (HBsAg-negative). There was no significant difference between patients receiving chemotherapy (group (A)) and polytransfused children [group (B)] (p=0.157), regarding the rate of HBV DNA. Anti-HBs ≥10 IU/L existed in 38.7% (12/31) of HBsAg positive patients and 49% (24/49) of HBV-DNA positive patients.

Summary/Conclusions: Children with neoplastic diseases vaccinated during infancy were at a high risk for HBV infection. The effect of immunosuppression on the HBV protective level favors overt HBV infection in children receiving chemotherapy. The co-existence of anti-HBs with HBsAg and/or HBV-DNA demonstrated a possible residual transfusion-transmission risk with mutant HBV strains.

PB2237

THE ISOHEMAGGLUTININ TITERS OF BLOOD BANK DONORS: THE EXPERIENCE OF ISTANBUL FACULTY OF MEDICINE

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Background: Isohemagglutinins that develop against ABO blood group antigens are very important in transfusion and transplantation medicine. Today, 30-40% of allogeneic stem cell transplantsations are ABO incompatible transplantation, 20-25% of which are major, 20-25% are minor and remaining bi-directionally incompatible transfusion. Our study; based on the knowledge that isohemagglutinins play an important role in blood transfusion policies in patients undergoing ABO incompatible hematopoietic stem cell transplantation (HSCT), has been shaped by the assumption that each healthy blood bank donor may be potential transfusion donors for ABO incompatible HSCT transplant recipients.

Aims: In this study, we investigated the isohemagglutinin titer values of the individuals with A, B and O blood groups; the distribution of the isohemagglutinin titters according to the decades and gender. Also we examined the possibility of determining the isohemagglutinin cut off value in Turkish society.

Methods: One thousand five voluntary blood donors (48 female, 957 male), randomly chosen from the donors, providing the criteria to be a standard blood donor in Blood Center Department, Istanbul Faculty of Medicine were studied. This study was approved by the Ethics Committee of Istanbul Medical Faculty. In the donor population group; blood group A (%40) was the most common and blood group AB was the rarest blood group. Approximately the Rh D phenotypes; 85% of the population was Rh D positive and 15% of the population was Rh D negative. The frequency of our blood group was determined similar with other European countries. The most common age range of one thousand five voluntary blood donors, including the same rate individuals with blood group A, B and O, was the age range between 26 and 35 years. Forward and reverse blood group determination were performed to these donors and also we identified the Anti-B Ig M and Ig G isohemagglutinin titer values for blood group A; Anti-A Ig M and Ig G titer values for blood group B; eventually both Anti-A Ig M / Ig G and Anti-B Ig M / Ig G isohemagglutinin titer values for blood group O by using column agglutination methods. Statistical analysis was performed with NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA).

Results: While the titer value of Anti-A Ig M isohemagglutinin was 1:128 for female individuals with blood group B; the titer values of both Anti-B Ig M (1:128 and 1:256) , Anti-B Ig G (1:1024) and Anti-A Ig M (1:256) isohemagglutinins were statistically significance in female individuals rather than male ones. The levels of isohemagglutinin in the blood groups A, B and O are shown in Table 1.A.B. There was no statistical difference in anti-B Ig G and Ig M titer in blood group A, anti-A Ig G and Ig M titer in blood group B and anti-A Ig G and Ig M titer in blood group O between males and females(p>0.05). However Anti-B Ig G and Ig M antibody titer values were higher in females than males in donors with blood group O respectively p=0.017 (p<0.05) and p= 0.001 (p<0.01) (Figure 1.A.B).

Table 1.

<table>
<thead>
<tr>
<th>Transfusion Therapy</th>
<th>Patients (N)</th>
<th>Hb Pre (g/dL)</th>
<th>Hb Post (g/dL)</th>
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Hb Pre: Pre-transfusion haemoglobin; Hb Post: Post-transfusion haemoglobin; PWC: Patients without post transfusion Hblevel; TPF: Total Patients Transfused; X- half haemoglobin.

PB2238

THE NEW METHOD OF PURIFICATION FACTOR COAGULATION VIII

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Background: The human plasma of blood can be transfused directly to patients or pooled and fractionated into plasma protein products. Plasma contains about 60-80 g/L of protein, of which about 95% are used for many therapeutic prod-
The use of eculizumab out of indication in typical HUS and whether the disease follow-up and to try to prevent possible relapses. Is to be noted the part played by the aproval of the product obtained by chromatography in the recovery of baseline renal function. Is to be noted the part played by the aproval of the product obtained by chromatography in the recovery of baseline renal function. Nevertheless, we consider that the approval of the product obtained by chromatography is one of the blood coagulation factor and it deficient causing development of bleeding disorders known as Haemophilia A. The purification of FVIII is generally required for the treatment Haemophilia A or von Willebrand’s disease and heavy loss of blood, requires relatively high purity for medical use. Aims: To review our experience in the management of the primary TMA and to raise a series of questions that perhaps could improve the approach of these pathologies. Methods: We made a retrospective, descriptive analysis of ten cases diagnosed of primary thrombotic microangiopathy (TTP n=5; typical HUS n=3; atypical HUS n=2) over the last eight years, 70% of which were women with an average age between 40-60 years. Only three cases had previous records of autoimmune diseases (MTCM, RA and HIV), all of which would eventually develop TTP. We made a retrospective, descriptive analysis of ten cases diagnosed of primary thrombotic microangiopathy (TTP n=5; typical HUS n=3; atypical HUS n=2) over the last eight years, 70% of which were women with an average age between 40-60 years. Only three cases had previous records of autoimmune diseases (MTCM, RA and HIV), all of which would eventually develop TTP. We requested ADAMTS13 levels on all cases, they were low (<5-10%) only in those patients diagnosed with TTP, and on the other hand confirming the positivity for Shiga toxin in those patients who eventually developed typical HUS. Methods: We made a retrospective, descriptive analysis of ten cases diagnosed of primary thrombotic microangiopathy (TTP n=5; typical HUS n=3; atypical HUS n=2) over the last eight years, 70% of which were women with an average age between 40-60 years. Only three cases had previous records of autoimmune diseases (MTCM, RA and HIV), all of which would eventually develop TTP. We requested ADAMTS13 levels on all cases, they were low (<5-10%) only in those patients diagnosed with TTP, and on the other hand confirming the positivity for Shiga toxin in those patients who eventually developed typical HUS. Results: Regardless of the diagnosis, 10-12 plasma exchanges were performed to improve the biological parameters of hemolysis, requiring the placement of a central catheter, most commonly at the right jugular vein (70%) due to the lower risk of thrombotic and infectious complications. Although renal involvement is frequent in HUS, only two of the patients required dialysis without recovery of baseline renal function. Is to be noted the part played by the approval of Active Scarlet Damask 4GT as ligands in combination methods of antiviral treatment. Results: The process plasma fractionation is largest industry segment in manufacture of therapeutic concentrate of plasma proteins. We developed technol- ogy scheme that involves fractionation plasma of blood in combinations of classical methods of protein precipitation and two chromatographic steps: ion exchange and affinity chromatography. Of all plasma fractionation methods, chromatography is the best candidate for purification of factor coagulation, especially FVIII. The methods adsorption/precipitation permits the fractionation of large volumes of plasma, but the quality of the product obtained by chromatography is superior. We offer: fresh frozen plasma – adsorption of proteins on the barium citrate – adsorption of proteins on Al(OH)3 – adsorption to proteins to PEG-4000 – viral inactivation (solvent-detergent method) – ion exchange chromatography on DEAE-Sepharose – viral inactivation (ammonium thiosinate) – dye-ligand affinity chromatography (Diasorob-Active Scarlet Damask 4GT). We got the drug of FVIII with specific activity 69.65±2.14 IU/mg protein.

Summary/Conclusions: We developed technological scheme of plasma fractionation and reached a high degree of purification of coagulation FVIII.

PB2239

PRIMARY THROMBOTIC MICROANGIOPATHIES. REVISION IN A CENTER OF THE LAST 8 YEARS

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1Fundación Jiménez Díaz, MADRID, Spain

Background: Thrombotic microangiopathies are a group of rare diseases characterized by non-immune microangiopathic hemolytic anemia, thrombocytope- nia and involvement of organs of varying intensity, mainly renal and CNS damage. TTP and HUS are the most important forms of TMA and without adequate treatment administered early are associated with high morbidity and mortality.

Aims: To review our experience in the management of the primary TMA and to raise a series of questions that perhaps could improve the approach of these pathologies

Methods: We made a retrospective, descriptive analysis of ten cases diagnosed of primary thrombotic microangiopathy (TTP n=5; typical HUS n=3; atypical HUS n=2) over the last eight years, 70% of which were women with an average age between 40-60 years. Only three cases had previous records of autoimmune diseases (MTCM, RA and HIV), all of which would eventually develop TTP. We requested ADAMTS13 levels on all cases, they were low (<5-10%) only in those patients diagnosed with TTP, and on the other hand confirming the positivity for Shiga toxin in those patients who eventually developed typical HUS.

Results: Regardless of the diagnosis, 10-12 plasma exchanges were performed to improve the biological parameters of hemolysis, requiring the placement of a central catheter, most commonly at the right jugular vein (70%) due to the lower risk of thrombotic and infectious complications. Although renal involvement is frequent in HUS, only two of the patients required dialysis without recovery of baseline renal function. Is to be noted the part played by the approval of Active Scarlet Damask 4GT as ligands in combination methods of antiviral treatment. Results: The process plasma fractionation is largest industry segment in manufacture of therapeutic concentrate of plasma proteins. We developed technol- ogy scheme that involves fractionation plasma of blood in combinations of classical methods of protein precipitation and two chromatographic steps: ion exchange and affinity chromatography. Of all plasma fractionation methods, chromatography is the best candidate for purification of factor coagulation, especially FVIII. The methods adsorption/precipitation permits the fractionation of large volumes of plasma, but the quality of the product obtained by chromatography is superior. We offer: fresh frozen plasma – adsorption of proteins on the barium citrate – adsorption of proteins on Al(OH)3 – adsorption to proteins to PEG-4000 – viral inactivation (solvent-detergent method) – ion exchange chromatography on DEAE-Sepharose – viral inactivation (ammonium thiosinate) – dye-ligand affinity chromatography (Diasorob-Active Scarlet Damask 4GT). We got the drug of FVIII with specific activity 69.65±2.14 IU/mg protein.

Summary/Conclusions: We developed technological scheme of plasma fractionation and reached a high degree of purification of coagulation FVIII.

PB2240

HAEMOVIGILANCE REPORTS OF ADVERSE BLOOD DONOR REACTION AMONG VOLUNTARY BLOOD DONORS IN TERTIARY CARE HOSPITAL IN KATHMANDU, NEPAL

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Background: Voluntary blood donation is widely considered to be safe with very minimum chance of adverse reaction, which may occur during or after the end of phlebotomy procedure.

Aims: To identify and understand the complication of adverse donor reactions though the incidence of reactions of blood donation among blood donor in the tertiary care hospital in Nepal.

Methods: This is a prospective study done among voluntary blood donors at Grande International Hospital, Kathmandu, Nepal from February 2013 to March 2015. The outlines of reported and communicated adverse donor reaction were also collected after the blood donation from voluntary blood donors in different locations including outdoor and in-house blood donation drive.

Results: In the present study 6,955 whole blood donors were included, during the period of 2 years, 105 (1.50%) adverse donor reactions were reported. Majority 89(84.76%) of adverse donor reactions were mild in nature such as, sweating; 27(25.72%), Light headnessed; 19(18.09%), nausea and vomiting; 15(14.28), allergy and bruises;11(10.47%), sore arm; 9(8.58%) and hematoma; 6(7.62%) while 16 (15.24%) were severe adverse reactions similarly, anaphy- lace;5(10.49%), loss of consciousness; 3(2.85%) and convulsive syncope;2(1.90%). Markers of the adverse donor reaction were age, sex, pulse, weight, blood pressure and donation status. Age and first time status were related with significantly higher risk of adverse reaction with 18-23 years old at higher risk compared to 24-55 years old. First time donors were at higher risk compared to repeated volunteer donors.

Summary/Conclusions: The results of the study are helpful to identify and understand the complication of adverse donor reactions though the incidence of reactions in the blood donor is lower than in other studies. Donor age and donation status were strong possibilities of complications.

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<td>S770, S797, S817, P245, P252, P589</td>
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<td>S807</td>
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<td>P285, P286</td>
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<td>Bobillo, S</td>
<td>P298, E995</td>
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<td>Bocchia, M</td>
<td>P163, P604, E941, E946, E1042, E1043, E1215, PB1818</td>
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<td>Boccomini, C</td>
<td>S778</td>
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<tr>
<td>Boeckh, M</td>
<td>S807</td>
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<td>Boeckx, B</td>
<td>E1220</td>
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<tr>
<td>Boeckx, N</td>
<td>P151</td>
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<td>Boenig, H</td>
<td>P382</td>
<td></td>
</tr>
<tr>
<td>Boerries, M</td>
<td>P350, E1355</td>
<td></td>
</tr>
<tr>
<td>Böettcher, S</td>
<td>S771</td>
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<tr>
<td>Bogdanović, A</td>
<td>E1328, PB1831</td>
<td></td>
</tr>
<tr>
<td>Bogh, M</td>
<td>P509, P607</td>
<td></td>
</tr>
<tr>
<td>Bogorodskaya, S</td>
<td>P220, E1133</td>
<td></td>
</tr>
<tr>
<td>Bok, I</td>
<td>E1238</td>
<td></td>
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<tr>
<td>Borsky, M</td>
<td>E1003, E1004</td>
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<tr>
<td>Borthakur, A</td>
<td>S148, S429, S436, P515</td>
<td></td>
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<tr>
<td>Borkhardt, A</td>
<td>S148, S429, S436, P515</td>
<td></td>
</tr>
<tr>
<td>Borsarelli Carvalho Brito, A</td>
<td>E952</td>
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<tr>
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<td>E1238</td>
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<tr>
<td>Borsky, M</td>
<td>E1003, E1004</td>
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<td>Bors, A</td>
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<td>Bordessoule, D</td>
<td>P609</td>
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<tr>
<td>Borg, K</td>
<td>E1220</td>
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<tr>
<td>Borley, S</td>
<td>E995</td>
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</tr>
<tr>
<td>Bornhauser, B</td>
<td>S437</td>
<td></td>
</tr>
<tr>
<td>Bornkamm, G</td>
<td>S809</td>
<td></td>
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<tr>
<td>Bos, G</td>
<td>S441, S501</td>
<td></td>
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<tr>
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<td>E1164</td>
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<tr>
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<td>E1441</td>
<td></td>
</tr>
<tr>
<td>Bosi, E</td>
<td>E1238</td>
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<td>Botta, A</td>
<td>E1222</td>
<td></td>
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<td>Botella, A</td>
<td>E1223, E1497</td>
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<td>P174</td>
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<td>Botelho, R</td>
<td>P706, E1320</td>
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<tr>
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<td>E829</td>
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<td>S779</td>
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Budeč, M, PB2140
Budziszewska, K, E920
Buechner, J, S476, P517
Bueno, D, E1563, PB1628
Bueno, J, E1541
Bueno, R, P361
Bueno, D, E1563, PB1628
Bueno, J, E1541
Bueno, R, P361
Bueren, J, S144, P229, P580, P623, P755, E1081, E1094, E1097, PB1839
Buffardi, S, E955
Buffiere, A, P514
Bug, G, P382
Bugatti, L, E1136
Bui, C, PB1699
Bui, D, P563
Bulder, I, S455
Bulian, P, P583, P588
Bullinger, L, P172, P182, P248, P533, E864, E868, E870, E887
Bulsa, J, PB1624
Bumbea, H, E1602
Bundschuh, R, P174, E883
Bunjes, D, E1084
Bunworasate, U, E1070
Buquicchio, C, PB1899
Burakov, V, P308
Buratti, J, E1368
Burchert, A, S424, P255
Burciu, A, S775
Burda, P, S483
Burgaleta, C, S544
Burger, J, S463, S769, S772, P240, P241, P588, E991, E1016
Burger, P, P132
Burgstaller, S, P558, P707
Burján, A, PB1627
Burke, J, P634
Burnelli, R, P715
Burnett, A, S475
Burney, C, P228
Burrows, F, P571, E834
Burthem, J, E1007
Burylev, V, E1153
Busca, A, P214
Busch, H, P243, P350, E1355
Büschel, G, P255
Buske, C, S429, S809, P635, E1246
Bussel, J, S435, P367, P723, P727, E1434, E1437
Bussolari, J, E1322
Bustaros, M, S779
Butler, A, E1201
Butters, M, E1481
Buttner, C, P527
Butylin, P, PB2047
Buzio, G, V, P707
Byk, J, P317
Bygrave, C, P374, E1245
Bygaa, K, P596, E1071
Byrne, C, P338, P339
Byrne, J, S423
Byrne, K, P278
Bystry, V, S801, S803
Byun, J, PB2004

C
 Cabrall-Hierro, L, S428
Caballero, A, E963, PB2159
Caballero, D, P746, E1129, E1169
Caballero, J, E1169, E1543
Caballero, M, P747, E1543
Caballero, T, P543
Caballero Berrocal, J, P318
Caballero-Velázquez, T, P213, P756
Cabanans-Perianes, V, E1562, PB1969, PB2033
Cabezón, M, P696, E831
Cabrera, C, S409, P688
Cabrera, J, E972, E1541, PB1885
Cabrero, M, P318, P655, E1169, E1592
Cacace, F, PB1707
Caccavelli, L, P631
Cacciagiù, S, E1123
Cacciola, E, P358, PB2040
Cacciola, R, P358, PB2040
Cadenas, B, E1156
Cadiere, B, PB2136
Cadiere, G, PB2136
Cadievski, L, E1471
Caieiro, G, E1508
Caers, J, E1250
Cafforio, L, PB1779
Cagol, D, E197, E921
Cagol, P, E1070
Cahn, J, E1178
Caiado, F, E889
Caiado, F, S410, PB2040
Caiarelli, P, S469, P633
Caiross, D, P407, S781
Calvo-R, S111, P722, E1195, E1443, PB1901, PB1931
Calvo, A, E1226
Çakıl, A, PB1748
Cakmakli, H, PB2021
Calabrese, C, P658
Calabretto, G, E1357, E1397
Calabria, A, S128
Calabuig, M, S790
Calado, D, S125
Calafiore, V, S111, PB2063
Calamar, D, PB2092
Calamar-Popovic, D, PB2124
Calisan, M, S461, P198, P205, P696, E1027
Calbi, V, S128
Caldes, C, P713
Caldron-Cabrera, C, S804, P318
Caldini, A, E1270
Caldora, M, P393
Caliskan, E, E1165, PB2123
Caliskan, Ü, E1584, PB1927
Calistri, D, E904
Calistri, E, S485, P604
Call, J, P633
Call, T, P254
Callahan, J, P699, P701, E1343
Callinan, M, E1380
Calle Primo, C, PB1879
Calle, MC, D, E1051
Callegari, B, PB2076, PB2077
Calleja, S, PB2082
Calvet-Buchau, E, E1024
Callum, J, S784
Calvins, B, P183, E1549
Calpadi, C, P280
Calpe, E, E1002
Calvaruso, G, P290
Calvillo, M, P234, P357, E1440
Calvo, J, S140
Calzamiglia, T, P667
Camacho, L, P694
Cametti, G, P667
Camilette, G, E1214
Camos, M, P168
Campagna, A, P1696
Campagna, G, P277
Campana, P, PB1957
Campati, A, E1136
Campagni, N, E1223
Campbell, H, P668
Campbell, P, E1201
Campbell-Drew, M, PB2045, PB2051
Deplano, S, S817
Depreter, B, E1114
DeRome, M, P322
Derreal, A, E1018
DerSarkissian, M, PB1825
Desai, P, PB1884
Deschamps, M, E1073
Deshet-Unger, N, E1107
Deshpande, N, E837, E843
Desmet, K, PB2152
Desmier, D, PB1987
Desmond, R, E1460, E1187, PB1962
Devadasan, D, E1484
Deveza, M, E1010
Devillet, B, P230
Devereux, S, P245, P589, E1509
Devetzoglou, M, PB1950, PB1961
Di Bartolomeo, P, P394, P738, PB1953, PB2059
Di Bella, N, P249
Di Benedetto, A, E1379
Di Carlo, E, E1226
Di Ciaccio, P, PB2014
Di Florio, F, E1167
Di Gaetano, R, PB2076, PB2077
Di Genua, C, S421
Di Giacomo, D, E1174
Di Gioia, M, PB1686, PB1983
Di Grazia, C, S796, E915, E1505
Di Ianni, M, P934
Di Marco, F, P717
Di Matola, T, E1570
Di Micco, A, P343, E1250
Di Noi, M, E1046
Di Palma, F, E873
Di Paolo, A, E1048
Di Piazza, F, PB2038, PB2039, PB2056
Di Persio, L, S437, E884
Di Raimondo, F, P858, E1043, E1214, E1232, E2063
Di Renzo, N, P81722, P8177
Di Rocco, A, P304, P81880
Di Rocco, M, E1077
Di Ruscio, A, P8164
Di Serio, C, S806
Di Tomaso, E, P188, E884
Di Tranl, M, P282
Di Tucci, A, P667, E1186
Di Tulio, A, P264, P657
Di Veroli, A, E908, E1340
Di Vita, A, P599, E1048
Diaa, N, PB2097
Diagne, I, E1495
Diamantopoulos, P, E1120, E1462
Diamond, J, S426
Diaa, G, PB264
Dias, J, E420
Diaz, L, PB1888
Diaz, M, PB1986, PB2022, PB2157
Diaz, V, PB1888
Diaz-Beya, M, S790, P202
Diaz de Heredia, C, S144, P580, P623
Diaz Morfa, M, PB2064
Diaz Varela, N, E1327
Dickinson, M, E1202
Dickman, P, P330
Diu, A, PB2057
Diehl, V, S150, P275
Diels, J, P687, E1024
Diericks, D, P565
Dierlam, M, S150
Dietlein, M, S150
Dietmae, T, E1365
Dietz, A, E840
Dietzel, H, E1449
Diez, B, E623
Diez, J, E1113
Diez-Campemo, M, P316, P318, P655, P660, P751, E1169, E1181
Diez Gallarreta, Z, E1183
Diez-Martin, J, PB1653
Diez-Pastor, J, P8095
Dijk, A, P340
Diklić, M, E1316, E1319, PB2140
Dikme, G, PB2222
Dikov, T, PB1693, PB1828
Dilek, I, PB2112
Dimrikopoulou, A, S416, P639
Dimopoulou, K, P324
Dimopoulou, M, S130, E1120, E1485
Dinou, M, P255, E1120
Dinovski, A, E147, PB1775, PB1795, PB2062
Ding, W, P254
Dingli, D, P673, E1240, E1241
Dinic Uzurov, V, PB1745
Diokle, S, E1365
Dirnhofer, S, E1365
Djek, I, P324
Dimopoulou, M, S130, E1120, E1485
Dinou, M, P255, E1120
Dinovski, A, E147, PB1775, PB1795, PB2062
Dimrikopoulou, A, S416, P639
Dimopoulou, K, P324
Diokle, S, E1365
Diou, M, E1432
Divona, M, E908, P8169
Dixon, S, S441
Dizzadarevic, A, PB2078
Djekic, D, E1308, E1316, P2140
Djunic, Z, P81871
Djunic, I, PB1704
Djurajnovic, V, S443, E970, PB1708, PB1771, PB1793, PB1799, PB1862
Djurjevic, P, S443, PB1799
Dljouy, I, E1137
Dmitrieva, E, E1029
Dnosezyne, A, E1257
Dmytrenko, I, PB1822, PB2005
Dmytrenko, O, PB2005
Dmytruk, N, E1010
Do, K-A, P241
Do, T, E1263
Do, Y, P259, P569, E871, E1054, E1069, E1070, PB1933
Do Nascimento, J, E1426, PB1861
Dobashi, A, S124
Dobashi, N, P314
Dobay, M, S437
Dobbernack, V, S494
Gionfriddo, I, P173
Giordano, A, PB2019
Giordano, C, P277, E1125
Giordano, P, P365
Giorgino, T, P351
Giotopoulos, G, S136
Gioula, G, PB2190
Giraldo, MD, P, P702, E1051, E1134, E1333, E1334, E1410, E1412, PB1989, PB2085
Giralt, S, P740, P745, P748, E1252, E1532, E1954
Girard, R, E1495
Girschikofsky, M, P559
Gladding, D, PB1788, PB1794
Glader, B, S451
Gladun, D, PB2208
Glaser, N, P243
Glasmacher, A, PB1676
Glazanova, T, E986
Gleixner, K, P693, E1071
Glembotsky, A, P365
Glinshchikova, O, E1403, E1409
Glossmann, J-P, E1251
Gluckman, E, E1495, PB2157
Gmati, G, E1514, PB2072
Gnan, C, P365
Gniot, M, P602, P611
Go, R, P331, P673, E1240, E1274
Go, S-I, E968, PB1715
Go, W, S466, P522
Goardon, N, S140
Godfrey, L, P540
Gómez, E, E1604
Gómez, M, P703
Gómez, P, E1586
Gómez-Aguado, F, PB1896
Gonzalez, A, P1664, E1287, PB1911, PB1914, PB1938
Gonzalez, R, PB2133
Gonzalez, S, PB1902
González Barca, E, S470, E957, E1394, PB2106
Gonzalez-McQuire, S, E1272, E1298, PB1966
González-Méndez, L, E1222
Gonzalez-Murillo, A, P510, P513
González Pérez, M, E1327
González-Porras, J, P361, E1451, E1592, PB2106
Goldschmidt, H, S458, P334, E1251, E1253, E1269, PB1966
Golenkov, A, PB1943
Göllner, S, E927
Golobokov, A, P307
Golubeva, M, PB2226
Goma, W, S440
Gomáriz, Á, P266
Gomber, M, S436
Gomes, A, PB2186
Gomes, A, PB1735
Gomes, B, PB2167
Gomes, M, PB2184
Gomes, S, E1586
Gomez, C, PB2057
Gomez, C, P554, E1086
Gómez, E, E1604
Gómez, M, P703
Gómez, P, E831
Gomez, A, PB2133
Gomez, V, E1565
Gómez-Aguado, F, PB1896
Gomez-Almaguer, D, E1306, E1415
Gómez-Castañeda, E, S491
Gómez-De Leon, A, E1306, E1415
Gómez-Espuch, J, E953
Gómez-Lion, A, E1027
Gómez-Marlo, P, PB1616
Gómez Morales, M, P768
Gómez Ronco, M, E983
Gomez Segura, G, PB2159
Gonzales, A, PB1763
Gonzales, A, E1064, E1287, PB1911, PB1914, PB1938
Gonzales, J, E1586
Gonzalez, L, E1586
Gong, B, E911, E916
Gong, X, E911, E916
Gong, X, P182
Gong, Y, P224
Gong, Z, PB1625, PB1939, PB2074
Gonzales, W, P331, P673, E1240
Gonzaga, Y, PB1637
Gonzales, F, E876
Gonzalez, A, P554, PB1755
González, B, P376, P554
González, B, PB1628
González, E, PB1888, PB1997
Gonzalez-Farre, B, E1394
Gonzalez Garcia, M-E, P329
González-Gascón y Marín, I, E1038, PB1761
González-González, B-J, PB1680
González Huerta, A, E1530
Gonzalez-Izquierdo, J, PB1847
Gonzalez-Martín, J, E1327
González-Martínez, T, E1327
Gonzalez-McQuire, S, E1272, E1298, PB1964
González-Mendoza, L, E1222
González-Muro, S, E1530, PB2122
Gonzalez-Murillo, A, P510, P513
González Pérez, M, E1327
González-Porras, J, P361, E1451, E1592, PB2106

22nd Congress of the European Hematology Association

XXVIII | haematologica | 2017; 102(s2)
Kueenburg, E, P343, E1250
Kuendgen, A, E937
Kufer, P, E867, E877
Kugler, S, E1449
Kuhnert, G, S150
Kuhns, S, PB1849
Kühner, T, E1449
Kuklik-Roos, C, S809
Kukreti, V, P680
Kulagin, A, S498, P718
Kulibaba, T, E1154
Kuliczkowski, K, E1553, PB1695, PB2032
Kulikov, S, E836, E853, E860, E1067, E1227, E1436
Kulis, M, S117, P300, P325, E1225
Kulikovsky, A, E853
Kunik, A, E1329
Kumagai, T, P263
Kumar, A, E974
Kumar, A, E843
Kumar, A, E1442
Kumar, B, P186
Kumar, N, E974
Kumar, P, E974
Kumar, S, S101, S408, S460, P331, P338, P673, P675, E1240, E1241
Kumode, T, E1100
Kunig, A, E864
Kurahashi, S, PB1742
Kurita, N, E974
Kurs, J, E1135
Kusnetsova, S, PB1792
Kushchevyy, E, E1127
Kushwaha, R, P507, P511, P725, E833, PB1677
Kwak, C, P764
Kwak, D-H, P551, P561
Kwak, JY, P259, E954, E1054, E1062, E1069, E1070
Kwak, L, P635
Kwei, L, P672
Kwik, J, S451, P614
Kwiatkowski, J, S451, S814
Kwon, J, E1069
Kwon, M, E1113
Kwong, YL, E1392, P8176
Kyle, R, P331, P673, E1240
Kyrcz-Krzemien, S, P741, E1061
Kyriakaki, S, S150
Kyriakou, C, PB1976
Kyriakou, D, E1266, PB1975, P82107
Kyriakopoulou, L, PB1860
Kyrtsonis, C, PB1784
Kyrtsonis, M, S415, S416, P639, E1120, E1266
Kyrtsonis, M-C, E1304
L
La Cava, P, E1214, E1232
La Nasa, G, S413, P256
La Rocca, F, E1226, E1261
La Sala, E, S111
Laadem, A, S129, P666
Labate, C, P610
LaCasse, R, S1860
Labopin, M, S493, P797, S798, P379, P384, P388, E1506
Labotka, R, P338
Ladoucai, G, P82199
Laddaga, F, E1406
Ladetto, M, S778, P304, E1408
Ladmiral, C, S809
Ladj, N, P371
Ladogana, S, P717
Laetsch, T, S476, S477, P517
Lafage, M, E1361
Lafort, M, P400
Laganà, C, P610
Lago, M, PB1896
Lagos, K, PB1860
Laguna, E, P81744
Laharanee, E, E1001
Lahay, A, S453, P82150
Lahuerta, JI, S409, P329, P679, P688, E1234, E1278
Lai, G-M, PB1719
Lai, K, P526
Lai, S, P256
Lai, S-W, PB1719
Lai, Y-Y, P507, P511, P725, E833, PB1677
Lake, A, P296
Lakhtani, S, PB1680
Lakner, V, PB1819
Lakshimi, T, E1089, E1091
Lakhman, A, E1240
Lai, I, S492
Lala, J, PB1797
Lalayann, C, E1610, PB1698, PB2102
Lally, J, PB1915
Lam, A, P687, E1281
Lam, L, S142
Lamagn, M, P400
Lamanna, M, S144, P755
Lambilliotte, A, S131
Lambrechts, D, E1220
Lammens, T, P156, E1114
Lamparelli, T, S796
Lampropoulou, R, E1249, PB2107
Melillo, L, S111
Melior-Heinke, S, S497
Melqvist, U-H, S501
Melnichenko, V, P31870
Melnick, A, P297, P325, E1225
Melo, D, E1075
Melo, M, PB1811, PB1938
Meltzer, A, P394, P399, E1570, E1571, E1575, E1577, PB2153, PB2188
Meloni, A, P520
Meloni, G, P520
Melosur, A, E1401
Melpignano, A, S129, PB1722, PB1992
Melville, K, E1350
Melvin, C, P571
Memoli, M, PB1707
Mendek-Czajkowska, E, P741
Mendeleeva, L, E1227, E1295, E1545, PB2000, PB2164
Mendez-Huerta, M, PB2219
Méndez Navarro, G, E1551
Mendes, T, PB1735
Mendoza, M, P550
Menezes, R, P190
Meng, H, E1085
Meng, L, P356
Mengarelli, A, E1145, E1379
Menna, G, P715, PB1647
Mendeleeva, L, E1227, E1295, E1545, PB2000, PB2164
Menu, E, E1211, E1220
Menzl, I, E1045
Meral Guenge, A, PB1927
Merali, G, E1078
Mercadal, S, E831, E957, PB1616, PB1887
Mercer, A, P294, P525
Merce, T, E1317
Mercier, M, E1045
Merce, A, E1145, E1379
Merritt, B, E950
Mersch, E, E1161
Mertens, D, S116, P248
Merz, M, PB1966
Mesegue, M, P168
Messenge, Y, S808
Mestet, E, E1560
Mestarellet, F, PB2031
Mestre, A, PB1874
Metamori, E, PB2162
Metaxas, JY, P509
Metelli, M, P599, E1048, E1318
Metti, A, E1481
Mettivier, I, E967
Metzke, K, PB1856, PB1859
Metzke, K, P546, P656, E885
Metzler, G, PB2023
Metzler, M, E1137, E1391
Metzner, M, S791
Meunier, M, E1178
Meydan, C, P325, E1225
Meyer, LH, P508, E838
Meyer, M, E838
Meyer-Pannenart, V, S116
Meziani, L, P268
Mezzabotta, M, P667, E1332
Mezzasomma, F, P173
Mezzatesta, C, S437
Micaelli, L, E911, E916
Miano, S, P657
Miano, M, P234, P575, E1440
Mianulli, A, E946
Miao, L, E1152
Miao, M, E931, PB1635
Mialyl, N, S125, E1359, E1385
Micausi, C, P234, P575, E1440
Miccio, A, S147
Miccoci, R, PB1899
Miclei, R, E1533
Michaels, V, P274
Michaels, L, E1192
Michalak, S, E1079
Michalski, E, P280, PB2107
Michaluk, S, M415
Michalet, M, S131, S797, S798, P557
Michalova, K, PB1620, PB1936
Michalski, W, P162
Michaud, L, E1001
Michel, G, P716, PB2157
Michel, M, PB2083
Michelis, R, E1015
Michot, J-M, S673, E1018
Michova, A, PB1693
Micheviciute, O, E1311
Mikys, U, E1399
Micol, M, E1315
Mico, C, P520
Miccucci, G, P636, E1136
Middelburg, R, P312
Mildolo, M, E821
Milenik, M, PB1769
Miele, L, P645
Mieling, C, S127, E1355, E1364
Miglino, M, S413, P283, P667, E854, E915, E919, E933, E1505, PB1632
Miglioni, V, E430
Migone De Amics, M, P396
Mihai, F, E1602
Mihaljevic, B, S443, E970, PB1704, PB1708, PB1771, PB1793, PB1799, PB1862
Mihaylov, G, E1556, PB2009
Mikula, G, PB1378
Mikhael, J, S457, P675
Mikhael, N, E1411
Mikhalin, A, PB2080
Mikhalinov, I, PB1807
Mikhalinaova, E, E1545, PB1852, PB2176
Mikhalev, M, PB2035, PB2028
Mikhaltsova, E, E1559
Miklaya, E, E982, PB2232
Miklos, D, S466, S492, P522
Mikulakova, Z, PB1766
Milan, E, E1232
Milani, M, E898
Milani, P, E387, P684, E1243
Milani, R, S128
Milani, S, E1420
Milano, F, PB1733
Milanovic, R, P732
Mili, N, S443, PB1771, PB1793, PB1799, PB1831
Miiljovic, D, PB1824
Miji, P, E1328
Millacoy, D, E1604, PB1729
Milian, S, PB2133
Millar, C, PB1797
Miller, C, S773, S777, E1130
Miller, C, P233
Miller, J, S108, P197, E846, E847, E921, PB1689
Miller, K, P165
Miller, R, P276
Mills, K, P539, P697
Mills, K, E1224
Millsdine, D, P509
Mline, P, E540
Milojkovic, D, S423, S817
Milone, G, PB2133
<table>
<thead>
<tr>
<th>Name</th>
<th>ID Numbers</th>
</tr>
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<tbody>
<tr>
<td>Naik, S</td>
<td>S146</td>
</tr>
<tr>
<td>Naima, M</td>
<td>E1472</td>
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<td>Naima, Z</td>
<td>PB2126</td>
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<td>PB1703, PB1855, PB2220</td>
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<td>Naitoh, C</td>
<td>PB2161</td>
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<tr>
<td>Najet, S</td>
<td>E1275, E1479, PB2126</td>
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<td>Najib, D</td>
<td>E1034, PB1781, PB1789</td>
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<tr>
<td>Nakagawa, M</td>
<td>S120</td>
</tr>
<tr>
<td>Nakagawa, Y</td>
<td>P263</td>
</tr>
<tr>
<td>Nakagawa, H</td>
<td>E1962</td>
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<td>Nakagawa, Y</td>
<td>P263</td>
</tr>
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<td>E1213</td>
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<tr>
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<td>PB2158</td>
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<td>PB1630, PB1716</td>
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<td>E1230, PB2126</td>
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<td>S492</td>
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<td>P691</td>
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<td>Nakao, S</td>
<td>P314</td>
</tr>
<tr>
<td>Nakashima, M</td>
<td>P383</td>
</tr>
<tr>
<td>Nakasone, H</td>
<td>P621</td>
</tr>
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<td>Nakatouki, I</td>
<td>E1227</td>
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<td>Nakauchi, H</td>
<td>E951</td>
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<tr>
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<td>PB1711</td>
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<td>Nakazato, T</td>
<td>E1058, E1066</td>
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<tr>
<td>Nakazawa, H</td>
<td>S120</td>
</tr>
<tr>
<td>Nakazawa, Y</td>
<td>P171</td>
</tr>
<tr>
<td>Nakry, T</td>
<td>P649</td>
</tr>
<tr>
<td>Nalçaci, M</td>
<td>PB2043</td>
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<tr>
<td>Nam, J</td>
<td>P680</td>
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<td>Nam, J</td>
<td>P732</td>
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<td>Naman, H</td>
<td>P217</td>
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<td>E950</td>
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<td>E1430</td>
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<td>PB1657</td>
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<td>E1000</td>
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<td>Narita, A</td>
<td>S120</td>
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<td>E1285</td>
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<td>S778</td>
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<td>E837</td>
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<tr>
<td>Nasimento, T</td>
<td>E1080, PB1904</td>
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<tr>
<td>Nasimento Costa, J</td>
<td>E1287, PB1914</td>
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<tr>
<td>Naseem, S</td>
<td>PB1805</td>
</tr>
<tr>
<td>Nassar, K</td>
<td>S777</td>
</tr>
<tr>
<td>Nasserinejad, K</td>
<td>S411, P340</td>
</tr>
<tr>
<td>Nasso, D</td>
<td>P384</td>
</tr>
<tr>
<td>Nasta, S</td>
<td>P566</td>
</tr>
<tr>
<td>Nestoupil, L</td>
<td>S772</td>
</tr>
<tr>
<td>Natale, A</td>
<td>P394</td>
</tr>
<tr>
<td>Natalija, B</td>
<td>E1110</td>
</tr>
<tr>
<td>Natalya, M</td>
<td>P311</td>
</tr>
<tr>
<td>Nathywani, A</td>
<td>P252</td>
</tr>
<tr>
<td>Natori, K</td>
<td>PB1731</td>
</tr>
<tr>
<td>Naughton, T</td>
<td>S802, P160</td>
</tr>
<tr>
<td>Naumann, N</td>
<td>E1344</td>
</tr>
<tr>
<td>Naumovska, Z</td>
<td>E1471</td>
</tr>
<tr>
<td>Nava, I</td>
<td>E1576</td>
</tr>
<tr>
<td>Nave-Gómez, C</td>
<td>PB1634</td>
</tr>
<tr>
<td>Navada, S</td>
<td>S488, E1170, E1185</td>
</tr>
<tr>
<td>Navaele, L</td>
<td>S466, P522, P523, E840</td>
</tr>
<tr>
<td>Navarro, A</td>
<td>S115, E1032</td>
</tr>
<tr>
<td>Navarro, A</td>
<td>PB1633</td>
</tr>
<tr>
<td>Navarro, B</td>
<td>E1541</td>
</tr>
<tr>
<td>Navarro, B</td>
<td>E972, PB1885</td>
</tr>
<tr>
<td>Navarro, B</td>
<td>S115</td>
</tr>
<tr>
<td>Navarro, J</td>
<td>E1394, E1396</td>
</tr>
<tr>
<td>Navarro, M</td>
<td>PB1887</td>
</tr>
<tr>
<td>Navarro, S</td>
<td>E1097, PB1839</td>
</tr>
<tr>
<td>Navas, B</td>
<td>P554</td>
</tr>
<tr>
<td>Nazarova, E</td>
<td>E1526, PB1945</td>
</tr>
<tr>
<td>Nazha, A</td>
<td>P352</td>
</tr>
<tr>
<td>Nazir, A</td>
<td>PB1718</td>
</tr>
<tr>
<td>Ndoro, S</td>
<td>PB2142</td>
</tr>
<tr>
<td>Ndreu, R</td>
<td>PB2188</td>
</tr>
<tr>
<td>Nebreda, Á</td>
<td>P536</td>
</tr>
<tr>
<td>Nebro Luque, M</td>
<td>PB1879</td>
</tr>
<tr>
<td>Nedopytanska, N</td>
<td>PB2088</td>
</tr>
<tr>
<td>Nee, J</td>
<td>P279</td>
</tr>
<tr>
<td>Neelapu, S</td>
<td>S466, P522</td>
</tr>
<tr>
<td>Neff, F</td>
<td>S809</td>
</tr>
<tr>
<td>Negoro, A</td>
<td>PB2173</td>
</tr>
<tr>
<td>Negrín, R</td>
<td>E1569</td>
</tr>
<tr>
<td>Neumann, D</td>
<td>E1107</td>
</tr>
<tr>
<td>Neuberger, D</td>
<td>P237</td>
</tr>
<tr>
<td>Neumann, D</td>
<td>E1107</td>
</tr>
<tr>
<td>Neumeister, P</td>
<td>E1363, E1395</td>
</tr>
<tr>
<td>Neureiter, D</td>
<td>P558</td>
</tr>
<tr>
<td>Nevado, J</td>
<td>PB1628</td>
</tr>
<tr>
<td>Neven, B</td>
<td>P716</td>
</tr>
<tr>
<td>Nevill, E</td>
<td>P581</td>
</tr>
<tr>
<td>Nevill, T</td>
<td>PB581</td>
</tr>
<tr>
<td>Newberry, K</td>
<td>P690</td>
</tr>
<tr>
<td>Newell, L</td>
<td>P210, P556</td>
</tr>
<tr>
<td>Newland, A</td>
<td>E1430, E1453</td>
</tr>
<tr>
<td>Newmark, J</td>
<td>E1149</td>
</tr>
<tr>
<td>Nezi, L</td>
<td>P321</td>
</tr>
<tr>
<td>Ng, H</td>
<td>E1413</td>
</tr>
<tr>
<td>Ngo, S</td>
<td>PB2150</td>
</tr>
<tr>
<td>Ngoc Quynh, A</td>
<td>P654</td>
</tr>
<tr>
<td>Nguyen, A</td>
<td>P287</td>
</tr>
<tr>
<td>Nguyen, C</td>
<td>P514</td>
</tr>
<tr>
<td>Nguyen, J</td>
<td>E1102</td>
</tr>
<tr>
<td>Nguyen, L</td>
<td>P186</td>
</tr>
<tr>
<td>Nguyen, N</td>
<td>P287</td>
</tr>
<tr>
<td>Nguyen-Khac, F</td>
<td>E1001, E1361</td>
</tr>
<tr>
<td>Nguyen-Quoc, S</td>
<td>E910</td>
</tr>
<tr>
<td>Ni, B</td>
<td>PB1994</td>
</tr>
<tr>
<td>Ni, Q</td>
<td>E1282</td>
</tr>
<tr>
<td>Ni Arnie, F</td>
<td>E1452</td>
</tr>
<tr>
<td>Niaz, G</td>
<td>E1572</td>
</tr>
<tr>
<td>Nibourel, O</td>
<td>E1171</td>
</tr>
<tr>
<td>Nickel, A</td>
<td>S497</td>
</tr>
<tr>
<td>Nicolae, C</td>
<td>PB1642</td>
</tr>
<tr>
<td>Nicolás, M</td>
<td>S444</td>
</tr>
<tr>
<td>Nicolas-Vrelizer, E</td>
<td>P572</td>
</tr>
<tr>
<td>Nicolau da Silva, M</td>
<td>PB1820</td>
</tr>
<tr>
<td>Nicolini, F</td>
<td>P257, P603</td>
</tr>
<tr>
<td>Nicolini, M</td>
<td>P636</td>
</tr>
<tr>
<td>Nicolin, B</td>
<td>P214</td>
</tr>
<tr>
<td>Nicolo’ C</td>
<td>E1254</td>
</tr>
<tr>
<td>Nie, K</td>
<td>S478, P524</td>
</tr>
<tr>
<td>Nie, Y</td>
<td>S490</td>
</tr>
</tbody>
</table>
haematologica | 2017; 102(s2) | LXV

Madrid, Spain, June 22 – 25, 2017
Ruiz Arguelles, A, E1500
Ruiz Arguelles, M, E1500
Ruiz Arguelles, G, E1500, PB2219
Ruiz-Cabello, F, P696
Ruiz Delgado, G, E1500, PB2219
Ruiz Delgado, R, E1500
Ruiz García, E, PB1738
Ruiz-Heredia, Y, P697, E891, E899, PB1912
Ruiz-Llobet, A, P168
Ruiz Reyes, G, E1500
Ruiz-Xiville, N, S461, PB1816
Rukavitcyn, A, PB1865
Rukaivtce, O, PB1865
Ruland, J, S818
Rule, S, E1377
Rumi, E, P351, P357, E1337
Rumelt, C, E1393
Rupa-Matysek, J, E1079
Ruparelia, M, PB1836
Rupoli, S, P636, E1136
Rupp, J, E1393
Rusk, C, PB2038, PB2039, PB2056
Rusinov, M, E853, E680, PB1615
Rusinov, M, E836, E1227
Ruskin, A, PB1829
Rutten, A, P190
Ryan, J, P508
Ryan, K, S481
Ryan, R, S808, P210, P556, P740, E922
Ryan, R, E994
Rybalkova, L, P308, E1568
Rybicka-Ramos, M, P741
Rybak, J, E1553, PB2032
Rydzanicz, M, PB1802
Rydzek, J, S816
Rylanc, G, E1202
Rymikiewicz, G, P162
Ryo, NH, E871
Ryu, D-B, P757
Rzyzhak, O, E1127
Rzyzhikova, N, E1203, E1409
Rzynower, P, PB1766
S
Saad, H, PB1767
Saad, S, E1494, PB1674
Saadeh, C, PB2177
Saadoun, H, P649
Sawedra, S, PB1782
Sabanci, E, PB2086
Sabater-Leal, M, P759, P765
Sabatini, F, P658
Sabatino, M, P523
Sabirou, F, PB1987
Sacca, V, P644
Sacardi, R, PB1983
Saccenti, E, E1020
Sacchi, M, S422
Sacchi, N, S796
Sacco, A, E1354
Sacconi, A, E1379
Sachdeva, M, E987
Sadak, K, P292
Sadeghi, B, P379
Sadelfain, M, S143, S479
Sadjad, S, P258
Sadjaian, A, P1072
Sadodd, S, P617
Sadri, S, E1159, PB1701, PB1746, PB1759
Sadullah, S, S502, P276, PB2057
Sadylkova, N, P307
Saeed, B, P179, P206
Saeed, H, E1465
Saes, I, P567
Salez-Salin, A, E1327
Saenz-Perdomo, M, PB2174, PB2179
Safi, L, S486
Safra, G, PB1642
Safsanova, G, PB1803
Sag, R, P689
Saglo, G, S485, P599, P601, P605, E1060, PB1818
Sagou, K, E1516
Saguer, M, PB2111
Saha, C, P276
Saha, V, E1465
Sahakyan, L, PB1643
Sahin, D, S774
Sahin, F, E1525, PB1968
Said, Q, P285
Said, Z, PB2236
Sail, M, S817
Sakia, T, S422, E1062
Sail, K, P728, E1466
Sailard, C, P354
Sainati, L, E1489, E1491, PB2142, PB2145
Saint-Martin, J-R, S469
Sainz Pérez, J, PB1974
Saito, H, P592
Saito, K, P612, E1104
Saito, K, E1230, PB2318
Saitoh, A, P625
Saitoh, K, P592
Saitoh, T, E1447, E1607
Saji, H, E1068
Sakai, H, S120
Sakai, R, PB1871
Sakamaki, H, P263
Sakamoto, J, P263
Sakamoto, M, E1229
Sakata, S, S124
Sakata-Yanagimoto, M, P301, E1519
Sakayori, T, PB2042
Sakelari, I, P280, E1233, E1407, E1610, PB2166
Sakr, M, PB2103
Sakura, H, PB2024
Sakura, T, E1522
Sai, A, PB2087
Sala, A, E955
Sala, E, P544, PB1878
Salam Eddine, B, PB1942, PB2018
Salama, A, P270, P723, E1437
Salama, E, PB2236
Salama, H, E1514, PB2072
Salamero, O, S790, P202, P554, PB1668
Salaroubat, C, P316
Salar, A, PB1726, PB1874
Salaroglio, I, E999
Salaroli, A, PB2136
Salas, C, PB1888
Salas, C, PB1997
Salas, M, PB1727, PB2008
Salaverria, I, S115
Salazar, R, E1074
Salciguoglu, Z, E1418, PB1640, PB1649
Saleh, M, P723, E1437
Salehzadeh, S, S1048
Salek, C, E841
Stamatopoulos, B, P246, E994
Stamatopoulos, K, S461, P242, P244, P378, E997, E1407
Stamatoullas, A, P316
Stamou, A, E1462
Stamou, M, PB2197
Stamouli, M, P280
Stanganelli, C, PB1764
Staninova-Stojovska, M, PB1775
Stanislav, B, P311
Stanulla, M, S436, S437
Stanzani, M, PB1932
Starbatty, B, E1251
Star∞, J, P350, P711, E823, PB1620
Staser, K, S794
Stassen, M, P651
Stathis, A, P282
Statuto, T, E1261, PB1801
Stauder, R, E1186
Stavroula, K, E1485
Stavroulaki, A, PB1950
Stavroyianni, N, S461, E1407, PB1698
Stawinski, P, P594, PB1802
Steegmann, MD, J, P609, E1051, E1059
Steele, A, P587, E996
Steensma, D, E1192
Steeples, V, S122
Stefanikova, Z, PB2009
Stefanova-Petrova, D, E1155
Stefani, H, S476
Stefanzl, G, P693
Stefoni, V, P304
Stegelmann, F, S424, P700, E1335
Steidl, S, PB1768
Steidl, U, S135
Stein, A, S471, S793, P191, P201, P524
Stein, B, S141
Stein, B, P701, P699, E1343
Stein, E, P471, P201, P215, P553
Stein, H, S150
Steinbauer, E, P1934
Steinemann, D, P226
Steinfeld, T, S108, P197, E921, PB1689
Steinfeldt, J, P711, E823
Peter, S, P523, E840
Stoyaov, Z, PB1828
Strehl, M, S141
Stratford, P, P321
Strati, P, P241, PB1760
Stroyan, P, PB1854
Stroyan, J, PB1936, PB1964
Stroube, G, P754
Stroobant, M, P326, P343, P669, E1242, E1245, E1550
Strofford, J, P587
Strofford, J, P585
Streichert, T, E1117
Strembítka, N, E1127
Strenne, O, P161
Strickland, S, S110, S473, P210, P560, E922
Streicher, T, E1117
Strobl, H, S419
Stroppiano, M, E1077
Strobel, E, E947
Strollo, M, E1817
Strub, H, E1374
Struski, S, E1001, E1361
Styner, M, E565
Stuart, R, P556, E922
Stühler, G, S797
Stühmer, T, P206, PB1934
Stukalov, A, S133
Stunnenberg, H, P325
Stunnenberg, H, S117
Sturniolo, M, E1239
Stüssi, G, S797
Suárez González, J, E1113
Suárez-Cisneros, H, S115, E1381
Suárez-González, J, PB1853
Subbiah, V, E1345
Subbotina, T, E1329
Subirà, D, E1688, PB2022, PB2064
Subklewe, M, P546, E867, E877
Subocz, E, E1193
Suboriceva, B, E1133
Stizhak, N, E1153
Stock, W, P519
Stockler-Goldstein, K, P319, E1248
Stockley, T, P708, E1324
Stoecklin, G, P663
Stoilova, B, S140
Stojanovic, A, P1774
Stojanovik, A, E1471
Stojic, S, PB1745
Stokes, M, E944
Stokke, C, E1141
Stolosa, T, P594, PB1802
Stolyar, M, E1314, PB2028
Stözl, F, P326, E880
Stone, R, P471, P552, E868, E870, E883
Stos-Weic, T, E1329
Stopka, T, E843, E1166
Stoppan, AM, E1549
Storino, F, P586, E1390
Storino, M, P393
Stork, L, S422
Stork, M, E1290
Storti, G, S111
Storti, P, P321
Storti, S, P604
Stosch, J, P656
Stolus, M, E828, E1311, E1399
Stotjes, M, PB2141
Stournos, D, E1249
Stout, S, P523, E840
Stoyanova, Z, PB1828
Straehm, B, P350
Stratford, E, P716
Strati, P, P241, PB1760
Stratigiaki, M, PB1854
Streubel, E, E947
Streetly, M, P326, P343, P669, E1242, E1245, E1550
Strefford, J, P587
Strefford, J, P586
Streichert, T, E1117
Strickland, S, S110, S473, P210, P560, E922
Strickland, S, S110, S473, P210, P560, E922
Stiri, A, S407, S781
Stringer, J, S500
Strob, H, S419
Stroppiano, M, E1077
Strobel, E, E947
Stroyan, M, E1817
Strub, H, E1374
Struski, S, E1001, E1361
Stricker, M, E1435
Stuart, R, P556, E922
Stuchl∞, J, P711, E823
Stuhler, G, S797
Stühmer, T, P206, PB1934
Stukalov, A, S133
Stunnenberg, H, P325
Stunnenberg, H, S117
Sturniolo, M, E1239
Stüssi, G, P282
Styles, L, S492
Su, Y, E835, E842, E925, E1480, PB1633
Su, Y, PB1719
Suarez, A, PB2174, PB2179
Suarez, F, P631, E910, E1236, E1386
Suarez, M, PB1874
Suárez-González, J, E1113
Suárez-Cisneros, H, S115, E1381
Suárez-González, J, PB1853
Subbiah, V, E1345
Subbotina, T, PB2035
Subirà, D, E1688, PB2022, PB2064
Subklewe, M, P546, E867, E877
Subocz, E, E1193
Suboriceva, B, E1133

haematologica | 2017; 102(s2) | LXXV

Madrid, Spain, June 22 – 25, 2017
Late Breaking Oral Session

LB2600

This abstract is part of the Presidential Symposium

NOVEL SMALL MOLECULE INHIBITORS CO-TARGETING CK1A AND P-TEFb DISRUPT SUPER-ENHANCERS AND ERADICATE ACUTE MYELOID LEUKEMIA IN A MOUSE MODEL

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Background: Whereas p53 is mostly non-mutated in AML, various oncogenic pathways, frequently through enhancing the activity of its major antagonist Mdm2, suppress its activity. We have previously showed that genetic ablation of CK1α robustly activates p53 (doi:10.1038/nature09673). However, with no selectable CK1α inhibitors for in vivo use, the therapeutic value of CK1α inhibition in hematological malignancies cannot be validated.

Aims: To develop small molecule CK1α inhibitors and assess their effect in mouse models of human leukemia.

Methods: CK1α inhibitors were identified via cell-based screening based on p53 activation. We focused on a small class of pyrazole-pyrimidine scaffolds, which through extensive medicinal chemistry yielded derivatives with high affinity binding, validated by crystallography studies, potent CK1α inhibitory activity and a good pharmacokinetic profile. Anti-leukemic activity was assessed by oral treatment in mouse models of AML. MLL-AF9 and Bcr-Abl Blast Crisis Results: We first demonstrated the inhibitors’ anti-leukemic effect by single oral dose treatment, robustly inducing p53 activation and blast cell cytoreduction (Figure 1).

These inhibitors distinguished leukemic from normal hematopoietic stem cells: they did not affect normal hematopoietic CFUs, but eliminated leukemic CFUs at an IC50 <9nM. We tested the long-term oral therapeutic effects of the inhibitors in MLL-AF9 leukemia mice. Whereas all vehicle-treated mice succumbed to the disease within a month, 40-50% of inhibitor-treated mice survived with no signs of disease up to 5 months’ observation, nor had the surviving mice any sequela of long-term treatment; all had normal blood counts and normal organ morphology and histology. Long-term leukemia control with possible cure, attesting to eradication of LSCs and preservation of normal HPCs was demonstrated by transplanting leukemia-treated BM into lethally irradiated mice: all transplanted mice recovered and none showed any evidence of residual disease within 6 months. To elucidate the mechanisms by which the inhibitors distinguished leukemic from normal hematopoietic cells, we profiled the kinome affinity of the inhibitors and further studied their signaling effects in vitro and in vivo. We found that CK1α inhibitors having potent anti-leukemia activity are distinguished from less active analogues by their capacity to co-target CDK9 and suppress the RNA Pol II elongation factors (P-TEFb, CDK9-CyclinT1 complex). This property, validated by co-crystallography studies, enables the inhibitors to disrupt super-enhancers (SE), demonstrated by suppression of chromatin H3K27 acetylation and Brd4 association. As a result, transcription of SE-dependent major anti-apoptotic leukemia oncogenes including Mdm2, Bcl-2 and Mcl-1 was nearly abolished and inhibitor-treated leukemia cells underwent apoptosis. Strikingly, brief drug exposure (10mins in vitro, 2hrs in vivo) results in prolonged (24hrs) SE suppression. This unique property, which is at variance with the current occupancy-driven pharmaceutical paradigm, likely contributes to the dramatic therapeutic effect of co-targeting CK1α and P-TEFb in leukemia.

Summary/Conclusions: We developed a new class of small molecule inhibitors that co-target CK1α and P-TEFb. These inhibitors induce very rapid, robust activation of p53 in synergy with shutdown of leukemic super-enhancers, resulting in a lasting, powerful and specific anti-leukemic therapeutic effects in vivo, with cure potential.

LB2601

CRYPTIC INSERTIONS OF IMMUNOGLOBULIN LIGHT CHAIN ENHANCER REGIONS ACTIVATE CCND1 AND CCND2 IN CYCLIN D1-NEGATIVE MANTLE CELL LYMPHOMAS


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Background: Mantle cell lymphomas (MCL) are characterized by the primary translocation t(11;14)(q13;q32) involving CCND1 and IG genes in virtually all cases. Recently, a small subset of cyclin D1-negative (cyclin D1−) MCL has been recognized. About half of these cases have CCND2 gene rearrangements and overexpression of this gene. However, the primary oncogenic events in cyclin D1−/cyclin D2-MCL still remain elusive.

Aims: To identify potential mechanisms driving the pathogenesis of cyclin D1−/cyclin D2−MCL.

Methods: We investigated 66 cyclin D1−/SOX11+ MCL cases by a combination of fluorescence in situ hybridization (FISH), gene expression profiling by Affymetrix U133+2.0 and qPCR (n=51), and copy number arrays (n=47) (Agilent CGH 1M, Affymetrix Oncoscan and 500K). Six cases were investigated by genome-wide sequencing including 4 mate-pair whole-genomes, 4 whole exomes, and 1 whole-genome sequencing. The male/female ratio was 2.5:1 and median age at diagnosis 66 years.

Results: Most cyclin D1−/MCL (49/51, 96%) showed overexpression of other G1 cyclins: CCND2 in 33/36 (91%), CCND2 in 12/32 (48%), and median overexpression of both CCNE1 and CCNE2 in 43/43 (100%). CCND2 rearrangements were detected by FISH in 25/33 cases (76%) with CCND2 overexpression, but the remaining CCND2+ cases and those with CCND3 overexpression did not show CCND2, CCND3 and IG rearrangements using currently used break-apart probes. Interestingly, by using pair-whole-genome and whole-exome sequencing analyses we discovered cryptic insertions of IG light chain regions including the enhancer regulatory elements (2 IGK and 1 IGL) near CCND3 gene in the three cases with cyclin D3 overexpression. These rearrangements were confirmed by Sanger sequencing and FISH with specifically designed probes to recognize the IG light chain rearranged regions in the genome, using these probes we identified 6 additional cases with cryptic IGK-CCND3, as well as 3 cases with IGK-CCND2 juxtaposition in tumors with high levels of CCND3 and CCND2, respectively. Taken together, 74% and 18% cases corresponded to cyclin D2+ and cyclin D3+ MCL, respectively; whereas 6% showed overexpression of CCNE1 and CCNE2 without CCND2 rearrangements. The global genomic profile of 47 cyclin D1−/SOX11+ MCLs had G1 cyclin overexpression. The detection of these rearrangements with cryptic IG light chain loci associated rearrangement, consisting of cyclin D1−/cyclin D2+ and cyclin D3+ MCL, respectively; whereas 6% showed overexpression of CCNE1 and CCNE2 without CCND2 rearrangements. The global genomic profile of 47 cyclin D1−/SOX11+ MCLs had G1 cyclin overexpression. The detection of these rearrangements with
Background: 1q (1q21 gain) is a common high-risk subtype of multiple myeloma (MM), which drives MM progression, confers drug resistance, and correlates with inferior outcome. However, the molecular mechanism underlying the adverse prognostic roles of 1q remains largely unclear. Recently, 1q has been linked to hypoxia and resulting drug-resistant gene expression.

Aims: To understand the function and clinical significance of hypoxia-induced factor-1β (HIF-1β), a gene located in the 1q21 region, in 1q MM and hypoxic microenvironment.

Methods: The relationship between 1q or HIF-1β and Btz response or overall survival (OS) was analyzed in patients with newly-diagnosed MM (NDMM). Western blot and qPCR analyses were performed to determine expression of HIF-1β and other 1q21 genes in 1q+ vs 1q− drug-resistant MM cells, or under hypoxia. The function of HIF-1β was evaluated using genetic means and pharmacological inhibitors.

Results: In a cohort of 180 NDMM patients, median OS (mOS) was 29 and 43 months for cases with (w) or without (w/o) 1q (P=0.038), among which 24.3, 43.3, and 43.8 months for 1q copy number ≥3,=3, and=2 (P=0.030), respectively; whereas Btz-based therapy displayed a marked increase in response rate ≥VGP; it failed to improve mOS of 1q patients significantly (28.5 and 33.9 months for patients w or w/o Btz treatment, P=0.983); in contrast, Btz treatment dramatically prolonged mOS in patients w/o vs w 1q (53.7 and 28.5 months, P=0.016). To explore the molecular basis for the adverse effect of 1q on prognosis, expression of the 1q21 genes related to drug resistance was examined. Notably, robust expression of HIF-1β at protein level was found in 1q+ MM cells, while no difference observed in CKS1B, a biomarker widely used as a marker of Btreatment success in 1q MM, or PSMB4 and MCL-1. Further, analysis of additional 40 NDMM patients revealed that HIF-1β mRNA level was significantly higher in MM patients, compared to normal donors (n=5, P <0.005); analysis of the microarray database UAMS “Multiple Myeloma DataBase” (University of Arkansas) also showed that HIF-1β expression was higher with MM progression. In high (e.g., NF, MS, PR) vs low risk (e.g., CD1, CD2, CD4, HY, LB) P <0.05 subtypes, or in w 1q vs w/o 1q (P <0.001 for copy number ≥3), as well as correlated to shorter OS (P =0.027). In the in vitro study, HIF-1β was markedly upregulated in MM cells acquired drug-resistance against Btz and lenalidomide, while no changes observed in other 1q21 genes (e.g., PSMD4, CKS1B). Ectopic expression of HIF-1β in 1q− cells reduced sensitivity of Btz. Hypoxia (1% O2) or its chemical mimetic lactic acid induced HIF-1β expression and Btz resistance, an event reversed by shRNA knockdown of HIF-1β. Furthermore, hypoxia-induced HIF-1β expression was associated with activation of NF-κB, which was prevented by the IKK inhibitor parthenolide, leading to restoration of HIF-1β expression and overall survival of MM patients was examined by Kaplan-Meier analysis.

Summary/Conclusions: Together, these findings argue that HIF-1β represents a potential marker for risk stratification and prognostic prediction of MM patients, especially those with high-risk cytogenetics such as 1q. They also suggest that HIF-1β might play a critical role in drug resistance related to microenvironmental factors (particularly hypoxia) and 1q21 gain, therefore serving as a potential therapeutic target for development of agents or therapy to overcome intrinsic and acquired drug resistance in MM.

Background: Hematopoietic stem cell transplantation remains the best therapeutic option for blood malignancies. Acute graft versus host disease (aGVHD) is one of the main potentially fatal complications of this treatment with an incidence as high as 50%. The NK cell population has been extensively studied as a potential target for treatments, as these cells have the capacity to potentiate the graft versus leukemia effect with a minimum risk for graft versus host reactions. Indeed, the abundance of circulating NK cells has been inversely correlated with the probability to develop (aGVHD). CD69 is a C-type lectin expressed on the surface of certain immune cell progenitors as well as activated mature leukocytes. CD69+ NK cells were previously shown to eliminate tumour cells more effectively than WT NK cells.

Aims: We wished to examine whether CD69+ NK cells would have a higher cytolytic capacity against activated allogeneic T cells and whether this would lead to successful aGVHD prevention.

Methods: We took advantage of a fully allogeneic aGVHD mouse model in which wild type (WT) or CD69+ BALBc mice were lethally irradiated and reconstituted with C57BL6 HSCs and naive T cells. Results were confirmed by in vivo killing assays as well as by use of CD69 neutralizing antibodies. Mouse strains deficient in T cells, B cells and NK cells were used to establish the NK cells as the main cell line supporting the aGVHD phenomenon. Mass cytometry was employed for extensive phenotyping of WT and CD69+ NK cells and RNAseq analyses were used to elucidate the molecular mechanisms implicated.

Results: CD69+ mice were highly resistant to aGVHD and significantly more efficient at eliminating hyper-reactive allogeneic T cells in vivo. This phenotype was reproduced in WT mice treated with a CD69 neutralizing monoclonal antibody during disease induction. Mass cytometry analyses showed that NK cells lacking CD69 expression upregulated the Ly49D and Ly49G2 receptors, responsible for self/non-self discrimination. Further, expression of inhibitory receptors such as CD94/NKG2A was downregulated in CD69+ NK cells. Finally, in vivo data and RNAseq analyses indicated that CD69+ NK cells are resistant to apoptosis. Preliminary data on NK cell chimerism from HSCT patients indicate that host NK cells can persist shortly after conditioning and transplant, and could be targeted with anti-CD69 mAb to avoid clonal expansion of highly reactive donor T cells.

Summary/Conclusions: NK cells treated with anti-CD69 mAb show a higher capacity to eliminate hyper-reactive allogeneic T cells and confer resistance to aGVHD. This data could pave the way for novel therapeutic strategies to optimize allogeneic HSCT.
Background: CTL019 is an investigational chimeric antigen receptor (CAR) T-cell therapy with a high rate of durable complete responses (CRs) and a manageable safety profile in a previously reported single-center trial in adult patients (pts) with R/R DLBCL.

Aims: Results of a planned interim analysis of a single-arm, open-label, multicenter, global phase 2 trial of CTL019 in pts ≥18 y with R/R DLBCL (JULIET; NCT02445248) are reported.

Methods: Industry-manufactured CAR T cells were provided to pts at 27 centers on 4 continents using a global supply chain. Pts had received ≥2 lines of antineoplastic therapy, 3 (range, 2-7). 51% of pts had prior autoSCT. Centralized manufacturing was feasible. CTL019 was genotyped in 422 pts to identify known variants in the viral envelope, T-cell receptor (TCR) β, and CD3 family before infusion. Aims: To determine the transcription factors binding sites we used ChroMat Immunoprecipitation coupled with sequencing (ChIP-seq).

Results: Statistical analysis revealed that GATA2 display dominant and independent targeting activity during the early phases of hematopoietic transcription programs. In addition, reprogrammed fibroblasts reprogramided immunodifficient NSG mice and generate hematopoietic progeny of multiple lineages, including T-cells and myeloid cells. Mechanically, GATA2 display dominant and independent targeting activity during the early phases of hematopoietic transcription programs. Results: Windows 10.

Summary/Conclusions: Together, these findings uncover a collaborative TF interaction that specifies hematopoietic progeny.

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LB2605

INDUCTION OF HEMOGENIC REPROGRAMMING IN HUMAN FIBROBLASTS

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Background: Hematopoietic stem cell (HSCs) are multipotent stem cells capable of sustaining all mature blood cells throughout life. During development, HSCs arise directly from specialized endothelial cells called hematopoietic endothelial (HE) cells within the developing aorta-gonad-mesonephros (AGM) region, in a process termed endothelial-to-hematopoietic transition (EHT). However, despite extensive studies in various animal models, the genetic program driving human HSC emergence remains largely unknown. We have previously reported the generation of hematopoietic precursor cells from mouse fibroblasts with the expression of transcription factors in the AGM region, but no EHT (HE-Vcs). These TFs induce a dynamic, multi-stage hematopoietic process that progresses through an endothelial-like intermediate, recapitulating developmental hematopoiesis in vitro.

Aims: Here, to better understand the molecular events underlying human HE cell specification we expressed hematogenic TFs in human fibroblasts and mapped the TF binding sites at initial stages of reprogramming.

Methods: To determine the transcription factors binding sites we used ChroMat Immunoprecipitation coupled with sequencing (ChIP-seq).

Results: Windows 10.

Summary/Conclusions: Together, these findings uncover a collaborative TF interaction that specifies hematopoietic progeny.

LB606

BONE MARROW SITES DIFFERENTIALLY IMPRINT DORMANCY AND CHEMORESISTANCE TO T-CELL ACUTE LYMPHOCYTIC LEUKEMIA

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Background: T-cell acute lymphoblastic leukemia (T-ALL) is a disease of T-cell progenitors, which mainly affects children and young adults. Numerous genomic alterations such as NOTCH1/FBXW7 mutations, TLX1/2 overexpression or SILD-TAL deletion are known to induce survival, proliferation and differentiation block in T-ALL cells. Differences in the growth and cell cycle progression within 30 days of infusion. No deaths were attributed to CTL019.

Summary/Conclusions: This planned interim analysis of a global study of CTL019 in adults with R/R DLBCL confirms the high response rates and durable CRs observed in the previous single-center experience in a cohort of highly pretreated patients. Centralized manufacturing was feasible. CTL019 was generally well tolerated without instance of treatment-related mortality. CRS and other AEs could be effectively and reproducibly managed by appropriately trained investigators.

LB2605

INDUCTION OF HEMOGENIC REPROGRAMMING IN HUMAN FIBROBLASTS

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Aims: Here, to better understand the molecular events underlying human HE cell specification we expressed hematogenic TFs in human fibroblasts and mapped the TF binding sites at initial stages of reprogramming.

Methods: To determine the transcription factors binding sites we used ChroMat Immunoprecipitation coupled with sequencing (ChIP-seq).

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Index of authors

A
Alkalay I LB2600
Anak O LB2604
Andreadis C LB2604
Arcangeli M-L LB2606
Awasthi R LB2604

B
Bachanova V LB2604
Ballas A LB2603
Ballerini P LB2606
Baruchel A LB2606
Beà S LB2601
Beltran S LB2601
Ben-Neriah Y LB2600
Bishop MR LB2604
Borchmann P LB2604

C
Cahu X LB2606
Calvo J LB2606
Campos E LB2601
Chang B LB2605
Chen B LB2605
Cicot G LB2601

D
Dai Y LB2602
Daniel M LB2605
de Jong D LB2601
de Leval L LB2601
Delabesse E LB2606
Delabie J LB2601

E
Espinet B LB2601

F
Ferreira L LB2605
Ferry JA LB2601
Fink A LB2600
Fleury I LB2604
Foley SR LB2604
Fu K LB2601

G
Gao S LB2602
García de Soria VG LB2603
Gomes A LB2605
González-Farré B LB2601
Gutiérrez-Abril J LB2601

H
Ho PJ LB2604
Holte H LB2604
Hei ED LB2601
Hung E LB2600

J
Jaffe ES LB2601
Jäger U LB2604
Jaglowski S LB2604
Jin F LB2602

K
Kurochkin I LB2605

L
Lachmann A LB2605
Landman-Parker J LB2606
Law K LB2605
Leblanc T LB2606
Lemischka IR LB2605
Li D LB2600
Liu X LB2602
López-Otin C LB2601

M
Ma’ayan A LB2605
Magenua JM LB2604
Martin P LB2603
Martin-Garcia D LB2601
Matutes E LB2601
Maziarz RT LB2604
McGuirk J LB2604
Mercurio F LB2600
Mielke S LB2604
Minzel W LB2600
Moore KA LB2605
Muñoz-Calleja C LB2603

N
Navarro A LB2601

O
O’Connor SJ LB2601
Oren M LB2600
Ott G LB2601

P
Pacaud L LB2604
Papatsenko D LB2605
Pereira C-F LB2605
Pérez García Y LB2603
Pflumio F LB2606
Pikarsky E LB2600
Poglio S LB2606
Puente XS LB2601

Q
Quintanilla-Martínez L LB2601

R
Relano M LB2603
Ribera-Cortada I LB2601
Rosenwald A LB2601
Rymkiewicz G LB2601

S
Salaverría I LB2601
Salles G LB2604
Satija N LB2605
Schuster SJ LB2604
Siebert R LB2601
Sun J LB2602
Sun Y LB2602
Swerdlow SH LB2601

T
Tai F LB2604
Tam C LB2604
Torrents D LB2601
Tsilingsiri K LB2603

U
Uzan B LB2606

V
Vacca J LB2600
Valdés-Mas R LB2601
Venkatachalam A LB2600

W
Waller EK LB2604
Wang X LB2602
Wang Z LB2605
Weisenburger D LB2601
Westin J LB2604
Woroniecka R LB2601
Wu C LB2602

Y
Yang P LB2602
Ye L LB2602
Yu X LB2602